NOVEL HUMAN PROTEIN KINASES AND USES THEREFOR

FIELD OF THE INVENTION

The invention relates to novel protein kinase nucleic acid sequences and proteins. Also provided are vectors, host cells, and recombinant methods for making and using the novel molecules.

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Application No. 09/659,289, filed September 12, 2000, which claims priority to U.S. Provisional Application No. 60/182,059, filed February 11, 2000, both of which are hereby incorporated herein by referenced in their entirety.

BACKGROUND OF THE INVENTION

Phosphate tightly associated with a molecule, e.g., a protein, has been known since the late nineteenth century. Since then, a variety of covalent linkages of phosphate to proteins have been found. The most common involve esterification of phosphate to serine, threonine, and tyrosine with smaller amounts being linked to lysine, arginine, histidine, aspartic acid, glutamic acid, and cysteine. The occurrence of phosphorylated molecules, e.g., proteins, implies the existence of one or more kinases, e.g., protein kinases, capable of phosphorylating various molecules, e.g., amino acid residues on

proteins, and also of phosphatases, e.g., protein phosphatases, capable of hydrolyzing various phosphorylated molecules, e.g., phosphorylated amino acid residues on proteins.

Protein kinases play critical roles in the regulation of biochemical and morphological changes associated with cellular growth and division (D'Urso *et al.* (1990) *Science* 250:786-791; Birchmeier *et al.* (1993) *Bioessays* 15:185-189). They serve as growth factor receptors and signal transducers and have been implicated in cellular transformation and malignancy (Hunter *et al.* (1992) *Cell* 70:375-387; Posada *et al.* (1992) *Mol. Biol. Cell* 3:583-592; Hunter *et al.* (1994) *Cell* 79:573-582). For example, protein kinases have been shown to participate in the transmission of signals from growth-factor receptors (Sturgill *et al.* (1988) *Nature* 344:715-718; Gomez *et al.* (1991) *Nature* 353:170-173), control of entry of cells into mitosis (Nurse (1990) *Nature* 344:503-508; Maller (1991) *Curr. Opin. Cell Biol.* 3:269-275) and regulation of actin bundling (Husain-Chishti *et al.* (1988) *Nature* 334:718-721).

Protein kinases can be divided into different groups based on either amino acid sequence similarity or specificity for either serine/threonine or tyrosine residues. A small number of dual-specificity kinases have also been described. Within the broad classification, kinases can be further subdivided into families whose members share a higher degree of catalytic domain amino acid sequence identity and also have similar biochemical properties. Most protein kinase family members also share structural features outside the kinase domain that reflect their particular cellular roles. These include regulatory domains that control kinase activity or interaction with other proteins (Hanks *et al.* (1988) *Science* 241:42-52).

Extracellular-signal-regulated kinases/microtubule-associated protein kinases (Erk\MAPKs) and cyclin-directed kinases (Cdks) represent two large families of serine-threonine kinases (see Songyang *et al.*, (1996) *Mol. Cell. Biol. 16*: 6486-6493). Both types of kinases function in cell growth, cell division, and cell differentiation, in response to extracellular stimulae. The Erk\MAPK family members are critical participants in intracellular signaling pathways. Upstream activators as well as the Erk\MAPK components are phosphorylated following contact of cells with growth factors or hormones or after cellular stressors, for example, heat, ultraviolet light, and inflammatory cytokines. These kinases transport messages that have been relayed from the plasma

membrane to the cytoplasm by upstream kinases into the nucleus where they phosphorylate transcription factors and effect gene transcription modulation (Karin *et al.*, (1995) *Curr. Biol. 5*: 747-757). Substrates of the Erk\MAPK family include c-fos, c-jun, APF2, and ETS family members Elk1, Sap1a, and c-Ets-1 (cited in Brott *et al.*, (1998) *Proc. Natl Acad. Sci. USA 95*, 963-968).

Cdks regulate transitions between successive stages of the cell cycle. The activity of these molecules is controlled by phosphorylation events and by association with cyclin. Cdk activity is negatively regulated by the association of small inhibitory molecules (Dynlacht, (1997) *Nature 389*:148-152). Cdk targets include various transcriptional activators such as p110Rb, p107 and transcription factors, such as p53, E2F and RNA polymerase II, as well as various cytoskeletal proteins and cytoplasmic signaling proteins (cited in Brott *et al.*, above).

A protein has been isolated in *Drosophilia*, designated *nemo*, which has homology to Erk\MAPKs and Cdks. A mammalian homologue of *nemo*, designated NLK, has been reported (Brott *et al.*, above). This protein kinase autophosphorylates and localizes to a great extent in the nucleus. This protein showed homology to both families of kinases (Erk\MAPKs and Cdks). It did not possess the characteristic MAPK phosphorylation motif TXY in the conserved kinase domain VIII. It instead exhibited the sequence TQE resembling the THE sequence found in some Cdks.

More recently, it was shown that NLK could down-regulate HMG-domain-containing proteins related to POP-1. The signaling protein Wnt regulates transcription factors containing high-mobility group (HMG) domains to direct decisions on cell fate during animal development. In *C. elegans*, the HMG-domain-containing repressor POP-1 distinguishes the fate of anterior daughter cells from posterior daughter cells throughout development. Wnt signaling down-regulates POP-1 activity in posterior daughter cells, for example, E. Meneghini *et al.*, (1999) *Nature 399*: 793-797, show that the genes MOM-4 and LIT-1 were also required to down-regulate POP-1 not only in E but in other posterior daughter cells. MOM-4 and LIT-1 are homologous to the mammalian components of the mitogen-activated protein kinase (MAPK) pathway of TAK-1 (transforming growth factor beta activated kinase (and NLK) *nemo*-like kinase, respectively. MOM-4 and TAK-1 bind related proteins that promote their kinase activity.

The authors of the report concluded that a MAPK-related pathway cooperates with Wnt signal transduction to down-regulate POP-1 activity.

In a further report by the same group (Ishitani *et al*,(1999) *Nature 399*: 798-802), it was shown that the TAK-1-NLK-MAPK-related pathway antagonizes signaling between beta-catenin and transcription factor TCF. The Wnt-signaling pathway regulates developmental processes through a complex of beta-catenin and the T-cell factor/lymphoid enhancer factor (TCF\LEF) family of high-mobility group transcription factors. Wnt stabilizes beta-catenin which then binds to TCF and activates gene transcription. This signal pathway is conserved in vertebrates, *Drosophilia* and *C. elegans*. In *C. elegans*, MOM-4 and LIT-1 regulate Wnt signaling during embryogenesis. MOM-4 is homologous to TAK-1 (a kinase activated by transforming growth factor beta). LIT-1 is homologous to mitogen-activated protein kinase kinase kinase (MAP3K) and MAP kinase (MAPK)-related *NEMO*-like kinase (NLK) in mammalian cells. This raised the possibility that TAK-1 and NLK were involved in Wnt signaling in mammalian cells. The authors reported that TAK-1 activation stimulates NLK activity and down-regulates transcriptional activation mediated by beta-catenin and TCF. Injection of NLK suppressed the induction of axis duplication by microinjected beta-catenin in *Xenopus* embryos. NLK was shown to phosphorylate TCF\LEF factors and inhibit the interaction of the beta-catenin-TCF complex with DNA. Accordingly, the TAK-1-NLK-MAPK-like pathway was shown to negatively regulate the Wnt signaling pathway.

Protein kinases play critical roles in cellular growth. Therefore, novel protein kinase polynucleotides and proteins are useful for modulating cellular growth, differentiation and/or development.

SUMMARY OF THE INVENTION

Isolated nucleic acid molecules corresponding to protein kinase nucleic acid sequences are provided. Additionally amino acid sequences corresponding to the polynucleotides are encompassed. In particular, the present invention provides for isolated nucleic acid molecules comprising the nucleotide sequences encoding the amino

acid sequences shown in SEQ ID NOS:2, 5, 8, 11, 14 or 17. Further provided are kinase polypeptides having amino acid sequences encoded by the nucleic acid molecules described herein.

The present invention also provides vectors and host cells for recombinant expression of the nucleic acid molecules described herein, as well as methods of making such vectors and host cells and for using them for production of the polypeptides or peptides of the invention by recombinant techniques.

The kinase molecules of the present invention are useful for modulating cellular growth and/or cellular metabolic pathways particularly for regulating one or more proteins involved in growth and metabolism. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding kinase proteins or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of kinase-encoding nucleic acids.

Another aspect of this invention features an isolated or recombinant kinase protein and polypeptide. Preferred kinase proteins and polypeptides possess at least one biological activity possessed by the naturally-occurring kinase.

Variant nucleic acid molecules and polypeptides substantially homologous to the nucleotide and amino acid sequences set forth in the sequence listing are encompassed by the present invention. Additionally, fragments and substantially homologous fragments of the nucleotide and amino acid sequences are provided.

Antibodies and antibody fragments that selectively bind a kinase polypeptide and fragments are provided. Such antibodies are useful in detecting a kinase polypeptide as well as in modulating cellular growth and metabolism.

In another aspect, the present invention provides a method for detecting the presence of kinase activity or expression in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of kinase activity such that the presence of kinase activity is detected in the biological sample.

In yet another aspect, the invention provides a method for modulating kinase activity comprising contacting a cell with an agent that modulates (inhibits or stimulates) kinase activity or expression such that kinase activity or expression in the cell is modulated. In one embodiment, the agent is an antibody that specifically binds to kinase

protein. In another embodiment, the agent modulates expression of kinase protein by modulating transcription of a kinase gene, splicing of a kinase mRNA, or translation of a kinase mRNA. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of the kinase mRNA or the kinase gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant kinase protein activity or nucleic acid expression by administering an agent that is a kinase modulator to the subject. In one embodiment, the kinase modulator is a kinase protein. In another embodiment, the kinase modulator is a kinase nucleic acid molecule. In other embodiments, the kinase modulator is a peptide, peptidomimetic, or other small molecule.

The present invention also provides a diagnostic assay for identifying the presence or absence of a genetic lesion or mutation characterized by at least one of the following: (1) aberrant modification or mutation of a gene encoding a kinase protein; (2) misregulation of a gene encoding a kinase protein; and (3) aberrant post-translational modification of a kinase protein, wherein a wild-type form of the gene encodes a protein with a kinase activity.

In another aspect, the invention provides a method for identifying a compound that binds to or modulates the activity of a kinase protein. In general, such methods entail measuring a biological activity of a kinase protein in the presence and absence of a test compound and identifying those compounds that alter the activity of the kinase protein.

The invention also features methods for identifying a compound that modulates the expression of the kinase gene by measuring the expression of the kinase sequence in the presence and absence of the compound.

The invention also provides compounds identified by the screening methods described herein.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 A, B and C shows the 18477 nucleotide sequence (SEQ ID NO:1) and the deduced amino acid sequence (SEQ ID NO:2). The methionine-initiated open reading frame of human 18477 (without the 5' and 3' untranslated regions) extends from nucleotide position 52 to position 2688 of SEQ ID NO:1, not including the terminal codon (coding sequence shown in SEQ ID NO:3).

Figure 2 A, B, C, D and E shows the 3695 nucleotide sequence (SEQ ID NO:4) and the deduced amino acid sequence (SEQ ID NO:5). The methionine-initiated open reading frame of human 3695 (without the 5' and 3' untranslated regions) extends from nucleotide position 115 to 3723 position of SEQ ID NO:4, not including the terminal codon (coding sequence shown in SEQ ID NO:6).

Figure 3 A and B shows the 13302 nucleotide sequence (SEQ ID NO:7) and the deduced amino acid sequence (SEQ ID NO:8). The methionine-initiated open reading frame of human 13302 (without the 5' and 3' untranslated regions) extends from nucleotide position 383 to position 1456 of SEQ ID NO:7, not including the terminal codon (coding sequence shown in SEQ ID NO:9).

Figure 4 A and B shows the 2208 nucleotide sequence (SEQ ID NO:10) and the deduced amino acid sequence (SEQ ID NO:11). The methionine-initiated open reading frame of human 2208 (without the 5' and 3' untranslated regions) extends from nucleotide position 76 to position 1818 of SEQ ID NO:10, not including the terminal codon (coding sequence shown in SEQ ID NO:12).

Figure 5 A and B shows the 2193 nucleotide sequence (SEQ ID NO:13) and the deduced amino acid sequence (SEQ ID NO:14). The methionine-initiated open reading frame of human 2193 (without the 5' and 3' untranslated regions) extends from nucleotide position 17 to position 1273 of SEQ ID NO:13, not including the terminal codon (coding sequence shown in SEQ ID NO:15).

Figure 6 A and B shows the 2249 nucleotide sequence (SEQ ID NO:16) and the deduced amino acid sequence (SEQ ID NO:17). The methionine-initiated open reading frame of human 2249 (without the 5' and 3' untranslated regions) extends from nucleotide position 114 to position 2000 of SEQ ID NO:16, not including the terminal codon (coding sequence shown in SEQ ID NO:18).

Figure 7 A and B shows an analysis of the 18477 open reading frame for amino acids corresponding to specific functional sites and to predicted transmembrane domains of SEQ ID NO:2.

Figure 8 A and B shows an analysis of the 3695 open reading frame for amino acids corresponding to specific functional sites and to predicted transmembrane domains of SEQ ID NO:5.

Figure 9 shows an analysis of the 13302 open reading frame for amino acids corresponding to specific functional sites and to predicted transmembrane domains of SEQ ID NO:8.

Figure 10 A and B shows an analysis of the 2208 open reading frame for amino acids corresponding to specific functional sites and to predicted transmembrane domains of SEQ ID NO:11.

Figure 11 shows an analysis of the 2193 open reading frame for amino acids corresponding to specific functional sites and to predicted transmembrane domains of SEQ ID NO:14.

Figure 12 A and B shows an analysis of the 2249 open reading frame for amino acids corresponding to specific functional sites and to predicted transmembrane domains of SEQ ID NO:17.

Figure 13 shows an analysis of the 18477 amino acid sequence: α β turn and coil regions; hydrophilicity; amphipathic regions; flexible regions; antigenic index; and surface probability.

Figure 14 shows an analysis of the 3695 amino acid sequence: α β turn and coil regions; hydrophilicity; amphipathic regions; flexible regions; antigenic index; and surface probability.

Figure 15 shows an analysis of the 13302 amino acid sequence: α β turn and coil regions; hydrophilicity; amphipathic regions; flexible regions; antigenic index; and surface probability.

Figure 16 shows an analysis of the 2208 amino acid sequence: α β turn and coil regions; hydrophilicity; amphipathic regions; flexible regions; antigenic index; and surface probability.

Figure 17 shows an analysis of the 2193 amino acid sequence: α β turn and coil regions; hydrophilicity; amphipathic regions; flexible regions; antigenic index; and surface probability.

Figure 18 shows an analysis of the 2249 amino acid sequence: α β turn and coil regions; hydrophilicity; amphipathic regions; flexible regions; antigenic index; and surface probability.

Figure 19 depicts a hydropathy plot of human 18477. Relative hydrophobic residues are shown above the dashed horizontal line, and relative hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) and N glycosylation site (Ngly) are indicated by short vertical lines just below the hydropathy trace. The numbers corresponding to the amino acid sequence (shown in SEQ ID NO:2) of human 18477 are indicated . Polypeptides of the invention include fragments which include: all or a part of a hydrophobic sequence (a sequence above the dashed line); or all or part of a hydrophilic

fragment (a sequence below the dashed line). Other fragments include a cysteine residue or as N-glycosylation site.

Figure 20 depicts a hydropathy plot of human 3695. Relative hydrophobic residues are shown above the dashed horizontal line, and relative hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) and N glycosylation site (Ngly) are indicated by short vertical lines just below the hydropathy trace. The numbers corresponding to the amino acid sequence (shown in SEQ ID NO:5) of human 3695 are indicated . Polypeptides of the invention include fragments which include: all or a part of a hydrophobic sequence (a sequence above the dashed line); or all or part of a hydrophilic fragment (a sequence below the dashed line). Other fragments include a cysteine residue or as N-glycosylation site.

Figure 21 depicts a hydropathy plot of human 13302. Relative hydrophobic residues are shown above the dashed horizontal line, and relative hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) and N glycosylation site (Ngly) are indicated by short vertical lines just below the hydropathy trace. The numbers corresponding to the amino acid sequence (shown in SEQ ID NO:8) of human 13302 are indicated . Polypeptides of the invention include fragments which include: all or a part of a hydrophobic sequence (a sequence above the dashed line); or all or part of a hydrophilic fragment (a sequence below the dashed line). Other fragments include a cysteine residue or as N-glycosylation site.

Figure 22 depicts a hydropathy plot of human 2208. Relative hydrophobic residues are shown above the dashed horizontal line, and relative hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) and N glycosylation site (Ngly) are indicated by short vertical lines just below the hydropathy trace. The numbers corresponding to the amino acid sequence (shown in SEQ ID NO:11) of human 2208 are indicated . Polypeptides of the invention include fragments which include: all or a part of a hydrophobic sequence (a sequence above the dashed line); or all or part of a hydrophilic

fragment (a sequence below the dashed line). Other fragments include a cysteine residue or as N-glycosylation site.

Figure 23 depicts a hydropathy plot of human 2193. Relative hydrophobic residues are shown above the dashed horizontal line, and relative hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) and N glycosylation site (Ngly) are indicated by short vertical lines just below the hydropathy trace. The numbers corresponding to the amino acid sequence (shown in SEQ ID NO:14) of human 2193 are indicated . Polypeptides of the invention include fragments which include: all or a part of a hydrophobic sequence (a sequence above the dashed line); or all or part of a hydrophilic fragment (a sequence below the dashed line). Other fragments include a cysteine residue or as N-glycosylation site.

Figure 24 depicts a hydropathy plot of human 2249. Relative hydrophobic residues are shown above the dashed horizontal line, and relative hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) and N glycosylation site (Ngly) are indicated by short vertical lines just below the hydropathy trace. The numbers corresponding to the amino acid sequence (shown in SEQ ID NO:17) of human 2249 are indicated . Polypeptides of the invention include fragments which include: all or a part of a hydrophobic sequence (a sequence above the dashed line); or all or part of a hydrophilic fragment (a sequence below the dashed line). Other fragments include a cysteine residue or as N-glycosylation site.

Figure 25 shows expression of the 2208 protein kinase in various normal human tissues. Tissues in which the expression levels of the 2208 mRNA was determined include, from top to bottom, aorta, brain, breast, cervix, colon, esophagus, heart, kidney, liver, lung, lymph, muscle, ovary, placenta, prostate, small intestine, spleen, testes, thymus, thyroid, and vein.

Figure 26 shows expression of the 2193 protein kinase in various human tissues and cells. The tissues and cell lines analyzed for 2193 expression include, from left to

right: lung; kidney; brain; heart; colon; tonsil; liver; fetal liver; spleen; Th1 48 hr.; Th2 48 hr.; Hep G-2-A; Hep G2.2.15-A; CD14; CD14 LPS; stellate (resting stellate cells, deprived of serum for 72 hours); stellate/FBS (serum reactivated stellate cells); NHLF mock (normal human dermal fibroblasts); NHLF TGF 48 hr.; liver Fibrosis (NDR); (columns 20-23); Th0 6 hr.; Th2 6 hr.; CD19; Grans (normal human granulocytes); PBMC/mock (peripheral blood mononuclear cells); PBMC/PHA; PBMC/IL10, IL4; PBMC/IL FNg, TNFα; NHBE/mock (normal human bronchial epithelial); NHBE/IL 13-2; BM-MNC (bone marrow mononuclear cells); mPB $CD34^+$; ABM $CD34^+$ ($CD34^+$ cells from adult bone marrow); erythroid; Meg day 7 (human megakaryocytes); Meg day 10; Meg day 14; Neutrophil day 7; Neutrophil day 14; MBM CDH b^-/ $CD14^+$; BM/GPA (GPA^+ cells from human bone marrow); and, erythroid day 6.

Figure 27 shows expression of the 2193 protein kinase in human tissues and virus-infected cultured hepatocytes (HepG2).

Figure 28 shows chromosome mapping information for the 2208 kinase.

Figure 29 shows chromosome mapping information for the 2249 kinase in various cell types.

Figure 30 shows PSORT prediction of protein localization for the 18477 protein kinase.

Figure 31 shows PSORT prediction of protein localization for the 13302 protein kinase.

Figure 32 shows PSORT prediction of protein localization for the 2208 protein kinase.

Figure 33 shows PSORT prediction of protein localization for the 2193 protein kinase.

Figure 34 shows PSORT prediction of protein localization for the 2249 protein kinase.

Figure 35 depicts an alignment of the protein kinase domain of human 18477 with a consensus amino acid sequence derived from a hidden Markov model. The upper sequence is the consensus amino acid sequences (SEQ ID NOS:21 and 22), while the lower amino acid sequence corresponds to amino acids 35-180 and amino acids 740-835 of SEQ ID NO:2, respectfully.

Figure 36 depicts an alignment of the protein kinase domain of human 3695 with a consensus amino acid sequence derived from a hidden Markov model. The upper sequence is the consensus amino acid sequence (SEQ ID NO:23), while the lower amino acid sequence corresponds to amino acids 8-259 of SEQ ID NO:5.

Figure 37 depicts an alignment of the protein kinase domain of human 13302 with a consensus amino acid sequence derived from a hidden Markov model. The upper sequence is the consensus amino acid sequences (SEQ ID NOS:24 and 25), while the lower amino acid sequence corresponds to amino acids 141-184 and amino acids 223-315 of SEQ ID NO:8, respectfully.

Figure 38 depicts an alignment of the protein kinase domain of human 2208 with a consensus amino acid sequence derived from a hidden Markov model. The upper sequence is the consensus amino acid sequences (SEQ ID NOS:26 and 27), while the lower amino acid sequence corresponds to amino acids 156-174 and amino acids 266-501 of SEQ ID NO:11, respectfully.

Figure 39 depicts an alignment of the protein kinase domain of human 2193 with a consensus amino acid sequence derived from a hidden Markov model. The upper sequence is the consensus amino acid sequence (SEQ ID NO:28), while the lower amino acid sequence corresponds to amino acids 26-278 of SEQ ID NO:14.

Figure 40 depicts an alignment of the protein kinase domain of human 2249 with a consensus amino acid sequence derived from a hidden Markov model. The upper sequence is the consensus amino acid sequences (SEQ ID NOS:29, 30, and 31), while the lower amino acid sequence corresponds to amino acids 167-239, 379-523 and amino acids 564-582 of SEQ ID NO:17, respectfully.

Figure 41 shows 18477 and cyclin B1 expression in WI-38 synchronized cells over a 27 hour time course.

Figure 42 shows 18477 expression in normal human tissues. Tissues analyzed for 18477 expression include, from left to right: prostate, osteoclasts; liver; breast; skeletal muscle; brain; colon; heart; ovary; kidney; lung; vein; trachea; aorta; adipose; small intestine; thyroid; skin; testes; placenta; fetal liver; fetal heart; undifferentiated osteoblasts; differentiated osteoblasts; primary cultured osteoblasts; fetal spinal cord; cervix; spleen; thymus; tonsil; and lymph node.

Figure 43 shows Taqman expression data for 18477 in various Xenofriendly cell lines.

Figure 44 shows 18477 and cyclin B1 expression in colon cell lines.

DETAILED DESCRIPTION OF THE INVENTION

Eukaryotic Protein Kinase Nucleic Acid and Polypeptide Molecules

The isolated nucleic acid molecules of the present invention encode a eukaryotic protein kinase polypeptide. Eukaryotic protein kinases (described in, for example, Hanks *et al.* (1995) *FASEB J.* 9:576-596) are enzymes that belong to an extensive family of proteins that share a conserved catalytic core common to both serine/threonine and tyrosine protein kinases. There are a number of conserved regions in the catalytic domain of protein kinases. One of these regions, located in the N-terminal extremity of the

catalytic domain, is a glycine-rich stretch of residues in the vicinity of a lysine residue, which has been shown to be involved in ATP binding. Another region, located in the central part of the catalytic domain, contains a conserved aspartic acid residue which is important for the catalytic activity of the enzyme (Knighton *et al.* (1991) *Science* 253:407-414). Two signature patterns have been described for this region: one specific for serine/threonine kinases and one for tyrosine kinases.

Eukaryotic protein kinase polypeptides can include one of the following consensus sequences (SEQ ID NOS:19, 20, and 32, respectively):

[LIV]-G-{P}-G-{P}-[FYWMGSTNH]-[SGA]-{PW}-[LIVCAT]-{PD}-x [GSTACLIVMFY]-x(5,18)-[LIVMFYWCSTAR]-[AIVP]-[LIVMFAGCKR]-K [K binds ATP]

[LIVMFYC]-x-[HY]-x-D-[LIVMFY]-K-x(2)-N-[LIVMFYCT](3)
[D is an active site residue]

[LIVMFYC]-x-[HY]-x-D-[LIVMFY]-[RSTAC]-x(2)-N-[LIVMFYC](3)
[D is an active site residue]

The present invention is based, at least in part, on the identification of novel protein kinase nucleic acid and polypeptide molecules, which may play a role in, or function in, signaling pathways associated with cellular growth and/or cellular metabolic pathways, and/or a productive viral infection. These growth and metabolic pathways are described in Lodish *et al.* (1995) *Molecular Cell Biology* (Scientific American Books Inc., New York, N.Y.) and Stryer *Biochemistry*, (W. H. Freeman, New York), the contents of which are incorporated herein by reference.

The present invention identifies novel kinases given the following numerical designations: 18477, 3695, 13302, 2208, 2193, and 2249. A plasmid containing the nucleotide sequence encoding human 18477 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on April 19, 2000 and assigned Accession Number PTA-1775. A plasmid containing the

nucleotide sequence encoding human 2193 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on July 7, 2000 and assigned Accession Number PTA-2204. A plasmid containing the nucleotide sequence encoding human 2249 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on May 12, 2000 and assigned Accession Number PTA-1868. A plasmid containing the nucleotide sequence encoding human 3695 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number ______. A plasmid containing the nucleotide sequence encoding human 13302 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number ______. A plasmid containing the nucleotide sequence encoding human 2208 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number ______. These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. These deposits were made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The 18477 protein kinase can modulate phosphorylation in a wide variety of tissues and cells that include, but are not limited to, islets, skin, endothelium, breast, colon, liver, brain, heart, kidney, skeletal muscle, trachea, aorta, adipose tissue, small intestine, testes, placenta, fetal liver, fetal heart, cervix, thymus, tonsil, lymph node and lung clinical tumor samples. Further, the kinase is cell-cycle regulated in WI-38 cells (G2/M). Taqman data has been used to confirm array expression data showing 18477 expression upregulated in S-G2/M phase of synchronized WI-38 cells. The gene has been shown to be similar to CEK1 (*S. pombe*) and tumor-suppressor protein, warts (*D. melanogaster*). CEK1 has been implicated in anaphase progression in fission yeast. Warts is a homolog of human myotonic distrophy kinase and is required for the control of cell shape and proliferation. The gene has been mapped to human chromosome 10 with a syntenic chromosome mo18. GAAT13D02 (5cR) D10S593 (12.6cR) are flanking markers. Nearby mutations/loci include human-prostate adenocarcinoma 1; SCIDA,

severe combined immunodeficiency, ATHABASCAN type; IDDM10, diabetes mellitus, insulin-dependent, 10; RDPA, REFSUM disease with increased pipecolicacidemia; OB10, obesity, susceptibility to, on chromosome 10; mouse:AX, ataxia, TW, twirler; LAH, lanceolate hair; NIDD2, non-insulin dependent diabetes mellitus 2. Nearby known genes include MGA1, DNMT2, CREM, and CACNB2.

The 3695 protein kinase molecule is expressed in tissues or cells including, but not limited to, adrenal gland, blood, brain, endothelial tissue, fibroblasts, keratinocytes, kidney, liver, muscle, ovary, skin, thymus, thyroid, and osteoblasts. In addition, the gene is also expressed in lymphoma.

The 13302 protein kinase is expressed in tissues or cells including, but not limited to, adrenal gland, breast, colon, D8 dendritic cells, endothelial cells, fibroblasts, heart, keratinocytes, lung, muscle, natural killer cells, nerve cells, prostate, skin and T-cells. In addition, the gene is also expressed in colon to liver metastases and lymphoma. The gene maps to human chromosome 20 with a location between D20S199 and D20S181 (6.2-9cM).

The 2208 protein kinase is expressed in tissues or cells that include, but are not limited to, those human tissues shown in Figure 25. Expression has also been observed in the following tissues/cells: adrenal gland, astrocyte, B-cell, brain, breast, colon, colon to liver metastases, endothelial, esophagus, heart, hypothalamus, keratinocytes, liver, lymphoma, melanocyte, muscle, nerve, osteoblast, prostate, retina, salivary gland, skin, spinal cord, spleen T-cells, testis, thymus and thyroid. The gene has been mapped in various cell types. The mapping data is shown in Figure 28.

The 2193 protein kinase is expressed in tissues or cells including, but not limited to, those shown in Figures 26 and 27. In particular, expression of the gene is differentially regulated in virus-infected hepatocytes. The gene is also differentially expressed in fibrotic liver tissues.

As disclosed herein, 2193 protein kinase is involved in viral infection (e.g., highly expressed or differentially expressed). The protein kinase thus modulates the activity of one or more proteins involved in viral infection, cellular growth, or differentiation, e.g., HBV-infected cells. The protein kinase is capable of modulating the phosphorylation state of a kinase molecule or the phosphorylation state of one or more proteins involved

in viral infection, cellular growth, or differentiation, e.g., HBV-infected cells. See also Lodish *et al.* and Stryer, supra. Particularly, the 2193 kinase modulates phosphorylation in HBV virus-infected tissues and cells, such as liver.

The 2249 protein kinase is expressed in tissues or cells including, but not limited to, adrenal gland, blood, bone, bone marrow, brain, breast, colon, colon to liver metastases, endothelial, heart, keratinocytes, kidney, liver, spleen, lung, lymphocyte, lymphoma, mammary gland, megakaryocytes, muscle, natural killer, nerve, osteoblast, ovary, pituitary, placenta, prostate, spinal cord, T-cell, thymus, and thyroid. Gene mapping is shown in Figure 29.

In addition, kinases of the present invention are targets of drugs described in Goodman and Gilman (1996) *The Pharmacological Basis of Therapeutics* (9^{th} ed.) Hartman & Limbard Editors, the contents of which are incorporated herein by reference.

The kinases of the invention contain domains or motifs, identified by routine homology searching procedures, for example in ProDom, Pfam, Prosite, MEMSAT, and PSORT . Such analysis has identified a eukaryotic protein kinase domain for the kinases of the present invention. For general information regarding PFAM identifiers, PS prefix and PF prefix domain identification numbers, refer to Sonnhammer *et al.* (1997) *Protein* 28:405-420 and http//www.psc.edu/general/software/packages/pfam/pfam.html.

As used herein, the term "protein kinase domain" includes an amino acid sequence of about 18-252 amino acid residues in length and having a bit score for the alignment of the sequence to the protein kinase domain (HMM) of at least 8. Preferably, an protein kinase domain includes at least about 18-252 amino acids, about 95-145 amino acid residues, about 72-170 amino acids, or about 144-234 and has a bit score for the alignment of the sequence to the protein kinase domain (HMM) of at least 16 or greater. The protein kinase domain (HMM) has been assigned the PFAM Accession No. PF00069 (http;//pfam.wustl.edu/). An alignment of the protein kinase domain (amino acids 35 to 180 and amino acids 740-835 of SEQ ID NO:2; amino acids 8-259 of SEQ ID NO:5; amino acids 141-184 and 223-315 of SEQ ID NO:8; amino acids 156-174 and amino acids 266-501 of SEQ ID NO:11; amino acids 26-278 of SEQ ID NO:14; and amino acids 167-239, amino acids 379-523, and amino acids 564-582 of SEQ ID NO:17) of human kinase sequences of the invention with a consensus amino acid sequence derived

from a hidden Markov model is depicted in Figures 35-40.

In a preferred embodiment kinase polypeptide or protein has a "protein kinase domain" or a region which includes at least about 18-252, at least about 100-250 more preferably about 130-200 or 160-200 amino acid residues and has at least about 60%, 70%, 80%, 90%, 95%, 99%, or 100% sequence identity with an "protein kinase domain," e.g., the protein kinase domain of human 18477, 3695, 13302, 2208, 2193, and 2249.

To identify the presence of an "protein kinase" domain in a kinase protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against a database of HMMs (e.g., the Pfam database, release 2.1) using the default parameters (http://www.sanger.ac.uk/Software/Pfam/HMM_search). For example, the hmmsf program, which is available as part of the HMMER package of search programs, is a family specific default program for MILPAT0063 and a score of 15 is the default threshold score for determining a hit. Alternatively, the threshold score for determining a hit can be lowered (e.g., to 8 bits). A description of the Pfam database can be found in Sonhammer *et al.* (1997) *Proteins 28(3)*:405-420 and a detailed description of HMMs can be found, for example, in Gribskov *et al.* (1990) *Meth. Enzymol. 183*:146-159; Gribskov *et al.* (1987) *Proc. Natl. Acad. Sci. USA 84*:4355-4358; Krogh *et al.* (1994) *J. Mol. Biol. 235*:1501-1531; and Stultz *et al.* (1993) *Protein Sci. 2*:305-314, the contents of which are incorporated herein by reference.

In one embodiment, a kinase protein includes at least one transmembrane domain. As used herein, the term "transmembrane domain" includes an amino acid sequence of about 15 amino acid residues in length that spans a phospholipid membrane. More preferably, a transmembrane domain includes about at least 18, 20, 22, 24, 25, 30, 35 or 40 amino acid residues and spans a phospholipid membrane. Transmembrane domains are rich in hydrophobic residues, and typically have an α-helical structure. In a preferred embodiment, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acids of a transmembrane domain are hydrophobic, e.g., leucines, isoleucines, tyrosines, or tryptophans. Transmembrane domains are described in, for example, http://pfam.wustl.edu/cgi-bin/getdesc?name=7tm-1, and Zagotta W.N. *et al.* (1996) *Annual Rev. Neuronsci. 19*:235-63, the contents of which are incorporated herein by

reference.

In a preferred embodiment, a kinase polypeptide or protein has at least one transmembrane domain or a region which includes at least 18, 20, 22, 24, 25, 30, 35 or 40 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% sequence identity with a "transmembrane domain," e.g., at least one transmembrane domain of human kinase polypeptides of the invention (e.g., amino acid residues 209-225 and 761-778 of SEQ ID NO:2; amino acids 188-205 and 1172-1189 of SEQ ID NO:5; amino acids 244-261 of SEQ ID NO:8; amino acids 89-111, 277-293 and 436-454 of SEQ ID NO:11 and amino acids 61-83, 249-265, and 408-426 of the mature polypeptide of SEQ ID NO:11; and amino acids 57-73 of SEQ ID NO: 17).

In another embodiment, a protein kinase of the present invention includes at least one "non-transmembrane domain." As used herein, "non-transmembrane domains" are domains that reside outside of the membrane. When referring to plasma membranes, non-transmembrane domains include extracellular domains (i.e., outside of the cell) and intracellular domains (i.e., within the cell). When referring to membrane-bound proteins found in intracellular organelles (e.g., mitochondria, endoplasmic reticulum, peroxisomes and microsomes), non-transmembrane domains include those domains of the protein that reside in the cytosol (i.e., the cytoplasm), the lumen of the organelle, or the matrix or the intermembrane space (the latter two relate specifically to mitochondria organelles). The C-terminal amino acid residue of a non-transmembrane domain is adjacent to an N-terminal amino acid residue of a transmembrane domain in a naturally occurring protein kinase.

In a preferred embodiment, a protein kinase polypeptide or protein has a "non-transmembrane domain" or a region which includes at least about 1-350, about 200-320, about 230-300, and about 240-280 amino acid residues, and has at least about 60%, 70% 80% 90% 95%, 99% or 100% sequence identity with a "non-transmembrane domain", e.g., a non-transmembrane domain of human protein kinase(e.g., amino acids 1-208, 224-760, 779-879 of SEQ ID NO:2; amino acids 1-187, 206-1171, 1190-1203 of SEQ ID NO:5; amino acids 1-243, 244-260, 262-358 of SEQ ID NO:8; amino acids 1-88, 112-276, 294-435, 455-581 of SEQ ID NO:11; and, amino acids 1-56 and 74-629 of SEQ ID NO:17). In specific embodiments, a non-transmembrane domain is capable of catalytic

activity (e.g., kinase activity).

A non-transmembrane domain located at the N-terminus of a protein kinase polypeptide is referred to herein as an "N-terminal non-transmembrane domain." As used herein, an "N-terminal non-transmembrane domain" includes an amino acid sequence having about 1-350, preferably about 30-325, more preferably about 50-320, or even more preferably about 80-310 amino acid residues in length and is located outside the boundaries of a membrane. For example, an N-terminal non-transmembrane domain is located at about amino acid residues 1-208 of SEQ ID NO:2; amino acids 1-187 of SEQ ID NO:5; amino acids 1-243 of SEQ ID NO:8; amino acids 1-88 of SEQ ID NO:11; and, amino acids 1-56 of SEQ ID NO:17.

Similarly, a non-transmembrane domain located at the C-terminus of a protein kinase polypeptide is referred to herein as a "C-terminal non-transmembrane domain." As used herein, an "C-terminal non-transmembrane domain" includes an amino acid sequence having about 1-300, preferably about 15-290, preferably about 20-270, more preferably about 25-255 amino acid residues in length and is located outside the boundaries of a membrane. For example, an C-terminal non-transmembrane domain is located at about amino acid residues 779-879 of SEQ ID NO:2, amino acids 1190-1203 of SEQ ID NO:5, amino acids 262-358 of SEQ ID NO: 8, amino acids 455-581 of SEQ ID NO:11, amino acids 74-629 of SEQ ID NO:17.

A protein kinase molecule can further include a signal sequence. As used herein, a "signal sequence" refers to a peptide of about 20-80 amino acid residues in length which occurs at the N-terminus of secretory and integral membrane proteins and which contains a majority of hydrophobic amino acid residues. For example, a signal sequence contains at least about 12-25 amino acid residues, preferably about 30-70 amino acid residues, more preferably about 61 amino acid residues, and has at least about 40-70%, preferably about 50-65%, and more preferably about 55-60% hydrophobic amino acid residues (e.g., alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, or proline). Such a "signal sequence", also referred to in the art as a "signal peptide", serves to direct a protein containing such a sequence to a lipid bilayer. For example, in one embodiment, a protein kinase polypeptide contains a signal sequence of about amino acids 1-29 of SEQ ID NO:11. The "signal sequence" is cleaved during processing of the

mature protein. The mature protein kinase polypeptide corresponds to amino acids 30-581 of SEQ ID NO:11.

For the 18477 protein kinase, PSORT prediction of protein localization is shown in Figure 30 and predicts nuclear localization with a potential vacuolar targeting motif. Prosite pattern analysis shows a serine/threonine protein kinase active site signature. Search for complete domains in Pfam shows a high score to eukaryotic protein kinase domains and some homology to protein kinase C terminal domain.

For the 3695 protein kinase, Prosite analysis shows a serine/threonine protein kinase active site signature. Search for complete domains in Pfam shows a high score to eukaryotic protein kinase domains.

For the 13302 protein kinase, homology searches have shown that the gene is similar to rat NIPK neuronal cell-death-inducible putative kinase. PSORT prediction of protein localization is shown in Figure 31, with the highest localization to mitochondria. A search of complete domains in Pfam shows a high score to eukaryotic protein kinase domains.

For the 2208 protein kinase, PSORT prediction of protein localization is shown in Figure 32. The highest score is shown for localization to mitochondria. Prosite analysis shows a serine/threonine protein kinase active site signature. A complete search of domains in Pfam shows a high score to eukaryotic protein kinase domains.

For the 2193 protein kinase, PSORT prediction of protein localization is shown in Figure 33 with highest scores for nuclear and cytoplasmic localization. Prosite analysis shows a serine/threonine protein kinase active site signature. A search of complete domains in Pfam shows a high score with eukaryotic protein kinase domains.

For the 2249 protein kinase, PSORT prediction of protein localization is shown in Figure 34 with the highest score to nuclear localization with the feature of having a coiled coil. Prosite analysis shows a serine/threonine protein kinase active site signature. A search of complete domains in Pfam shows the highest homology with eukaryotic protein kinase domains.

The term "kinase" includes a protein, polypeptide, or other non- proteinaceous molecule that is capable of modulating its own phosphorylation state or the phosphorylation state of a different protein, polypeptide, or other non- proteinaceous

molecule. However, the term "kinase," when referring to the proteins or polypeptides of the invention, refers to a protein kinase, since the kinases of the invention are protein kinases.

Kinases can have a specificity for (i.e., a specificity to phosphorylate) serine/threonine residues, tyrosine residues, or both serine/threonine and tyrosine residues, e.g., the dual-specificity kinases. As referred to herein, kinases, such as protein kinases, preferably include a catalytic domain of about 200-400 amino acid residues in length, preferably about 200-300 amino acid residues in length, or more preferably about 250-300 amino acid residues in length, which includes preferably 5-20, more preferably 5-15, or most preferably 11 highly conserved motifs or subdomains separated by sequences of amino acids with reduced or minimal conservation. Specificity of a kinase for phosphorylation of either tyrosine or serine/threonine can be predicted by the sequence of two of the subdomains (VIb and VIII) in which different residues are conserved in each class (as described in, for example, Hanks *et al.* (1988) *Science* 241:42-52, the contents of which are incorporated herein by reference). These subdomains are also described in further detail herein.

Kinases play a role in signaling pathways associated with cellular growth. For example, protein kinases are involved in the regulation of signal transmission from cellular receptors, e.g., growth-factor receptors, entry of cells into mitosis, and the regulation of cytoskeleton function, e.g., actin bundling.

Assays for measuring kinase activity are well known in the art depending on the particular kinase. Specific assay protocols are available in standard sources known to the ordinarily skilled artisan. For example, see "Kinases" in Ausubel *et al.,* eds. (1994-1998) *Current Protocols in Molecular Biology* (*3*) and references cited therein; http://www.sdsc.edu/Kinases/pkr/pk_protocols.html; and http://www.sdsc.edu/Kinases/pkr/pk_protocols/tyr_synpep_assay.html

Inhibition or over-stimulation of the activity of kinases involved in signaling pathways associated with cellular growth can lead to perturbed cellular growth, which can in turn lead to cellular growth related-disorders. As used herein, a "cellular growth-related disorder" includes a disorder, disease, or condition characterized by a deregulation, e.g., an upregulation or a downregulation, of cellular growth. Cellular

growth deregulation may be due to a deregulation of cellular proliferation, cell cycle progression, cellular differentiation and/or cellular hypertrophy. Examples of cellular growth related disorders include cardiovascular disorders such as heart failure, hypertension, atrial fibrillation, dilated cardiomyopathy, idiopathic cardiomyopathy, or angina; proliferative disorders or differentiative disorders such as cancer, e.g., melanoma, prostate cancer, cervical cancer, breast cancer, colon cancer, or sarcoma. Disorders associated with virally-infected cells or tissues are also encompassed. The compositions are useful for the treatment of viral infection, such as DNA virus infection, including but not limited to HBV.

The 18477 protein kinase is expressed in islets, skin, endothelium, breast, colon, liver, brain, heart, kidney, breast, colon, skeletal muscle, trachea, aorta, adipose tissue, small intestine, testes, placenta, fetal liver, fetal heart, cervix, thymus, tonsil, lymph node and lung clinical tumor samples as discussed above. Expression of the gene is particularly relevant in cell cycle progression and cellular differentiation, and accordingly, is particularly relevant for development, differentiation, and carcinogenesis. The gene is expressed in clinical tumor samples including, but not limited to, breast, colon and lung. 18477 is a cell cycle regulated kinase. It has been shown to be upregulated 2-5 times in 9/11 colon tumors when compared to normal colon tissues (Figure 44). The gene is expressed in clinical tumor samples including, but not limited to, breast, colon and lung. Many cell cycle regulated genes may be considered protooncogenes. Cell cycle regulated genes control cell proliferation. Thus, modulation of these genes and their regulatory activities may permit the control of tumor cell proliferation and invasion. Accordingly, the disclosed invention relates to methods and compositions for the modulation, diagnosis and treatment of disorders associated with the tissues in which the gene is expressed, and particularly, in carcinogenesis, especially in breast, colon and lung.

The 2249 protein kinase is expressed in cells and tissues including, but not limited to, those listed hereinabove. Accordingly, the disclosed invention relates to methods and compositions for modulation, diagnosis and treatment of disorders associated with these cells and tissues. Because the gene is expressed in blood cells, in one embodiment, gene expression is relevant to treatment of immune and inflammatory disorders. Moreover,

because the gene is expressed in colon to liver metastases, in one embodiment expression is important in colon cancer and in colonic metastases to the liver. The gene is also expressed in keratinocytes, and accordingly, in one embodiment expression of the gene is relevant to the development of epithelium and other structures in which keratinocytes are precursor cells. Because the gene is also expressed in lymphoma, in one embodiment, gene expression is relevant to this disorder. Further, because the gene is expressed in megakaryocytes, expression of the gene is relevant to disorders associated with platelets, including thrombocytopenia. Further, because the gene is expressed in osteoblasts, in one embodiment expression is relevant to bone disorders that include defects in development of proper bone mass, and accordingly, is relevant in osteoporosis and osteopetrosis.

The 2193 protein kinase is expressed in tissues and cells, including those shown in Figures 26 and 27. Accordingly, the invention relates to methods and compositions for the modulation, diagnosis and treatment of disorders associated with these cells and tissues. Particularly relevant embodiments include disorders of those tissues in which the gene is highly expressed, such as brain and erythroid cells. Moreover, because the gene is highly expressed in $CD34^+$ cells, expression of the gene is relevant to all hematopoietic lineages, and therefore to anemia, neutropenia and thrombocytopenia. In addition, some expression of the gene is found in fibrotic liver tissue and thus, expression can be relevant to tissue fibrosis and particularly liver fibrosis. In this regard, Figure 27 shows increased expression of 2193 in Hep G2 cells that have been infected with hepatitis B virus.

RNA was isolated from HepG2 (immortalized human hepatocyte cells) and a HepG2 cell line stably transfected with the HBV genome. These cell lines can be used to screen for anti-HBV compounds. The RNA was labeled by synthesizing ^{33}P-labeled cDNA and hybridized to a gene array containing novel human genes identified by the inventors. 2193 RNA was found to be significantly more abundant in HBV-infected HepG2 cells than in uninfected HepG2 cells.

The disclosed invention accordingly relates to methods and compositions for the modulation, diagnosis, and treatment of disorders associated with, caused by, or related to viral infection. These disorders can manifest as immune, inflammatory, respiratory, hematological, cardiovascular, and other disorders including, but not limited to, AIDS,

virus associated leukemias, lymphomas, sarcomas, and carcinomas, herpetic infections and collateral symptoms, EBV infection, including mononucleosis, hepatitis virus infection, including A, B, C, and D viruses, viral induced liver cancer, and viral pneumonias.

Viruses include, but are not limited to, those identified with carcinogenesis, including hepatitis B virus (HBV) and liver cancer, Epstein-Barr virus (EBV), and lymphoma, human T-cell lymphotrophic virus Type I (HTLV-1) and leukemia, and human *Herpes* virus 8 (HHV-8) and Kaposi sarcoma. Virus families to which the invention pertains include but are not limited to Adenoviridae, Picornaviridae, Coronaviridae, Orthomyxoviridae, Paramyxoviridae, Reoviridae, Caliciviridae, Hepadnaviridae, Viroid-like, Flaviviridae, Norwalk-like, Togaviridae, Parvoviridae, Poxviridae, Herpesviridae, Retroviridae, Reoviridae (Orbivirus), Arenaviridae, Bunyaviridae, Filoviridae, Hantavirus, and Papovaviridae. Respiratory diseases have been associated with Adenovirus, Echovirus, Rhinovirus, Coxsackievirus, Coronavirus, Influenza viruses A, B, Parainfluenza virus 1-4, and Respiratory syncytial virus. Viral diseases of the respiratory system include, but are not limited to, lower respiratory tract infections, conjunctivitis, diarrhea; upper respiratory tract infections, pharyngitis, rash; pleurodynia, herpangina, hand-foot-and-mouth disease; influenza, croup, bronchiolitis, and pneumonia. Digestive diseases have been associated with Mumps virus, Rotavirus, Norwalk agent, Hepatitis A Virus, Hepatitis B Virus, Hepatitis D Virus, Hepatitis C Virus, and Hepatitis E Virus. These include but are not limited to mumps, pancreatitis, orchitis; childhood diarrhea; gastroenteritis; acute viral hepatitis; acute or chronic hepatitis; with HBV, acute or chronic hepatitis; and enterically transmitted hepatitis. Systemic viral pathogens associated with skin eruptions include, but are not limited to, Measles virus, Rubella virus, Parvovirus, Vaccinia virus, Varicella-zoster virus, Herpes simplex virus 1, and Herpes simplex virus 2. Disease expression includes, but is not limited to, Measles (rubeola); German measles (rubella); Erythema infectiosum, aplastic anemia; smallpox; chickenpox, shingles; "cold sore"; and genital herpes. Systemic viral pathogens associated with hematopoietic disorders include Cytomegalovirus, Epstein-Barr virus, HTLV-I, HTLV-II, HIV-1 and HIV-2. Disease expression includes, but is not limited to, Cytomegalic inclusion disease; infectious mononucleosis; adult T-cell

leukemia; tropical spastic paraparesis; and AIDS. Viral pathogens associated with Arboviral and Hemorrhagic fevers include, but are not limited, Dengue virus 1-4, yellow fever virus, Colorado tick fever virus, and regional hemorrhagic fever viruses. Disease expression includes, but is not limited to, Dengue, hemorrhagic fever; yellow fever; Colorado tick fever; Bolivian, Argentinian, Lassa fever; Crimean-Congo, Hantaan, sandfly fever; Ebola, Marburg disease; Korean, U.S. pneumonia. Viral pathogens associated with warty growths include Papillomavirus and molluscum virus. Disease expression includes, but is not limited to, Condyloma; cervical carcinoma; and molluscum contagiosum. Viral pathogens associated with diseases of the central nervous system include, but are not limited to, Poliovirus, Rabiesvirus, JC virus, and Arboviral encephalitis viruses. Disease expression includes, but is not limited to, Poliomyelitis; Rabies; progressive multifocal leukoencephalopathy (opportunistic); Eastern, Western, Venezuelan, St. Louis, California group.

The 3695 protein kinase is expressed in the tissues and cells disclosed hereinabove. The invention accordingly relates to methods and compositions for the modulation, diagnosis and treatment of disorders associated with these tissues and cells. The gene is also expressed in keratinocytes, and accordingly, in one embodiment expression of the gene is relevant to the development of epithelium and other structures in which keratinocytes are precursor cells. Because the gene is also expressed in lymphoma, in one embodiment, gene expression is relevant to this disorder. Further, because the gene is expressed in osteoblasts, in one embodiment expression is relevant to bone disorders that include defects in development of proper bone mass, and accordingly, is relevant in osteoporosis and osteopetrosis.

The 13302 protein kinase is expressed in the tissues and cells described hereinabove. Accordingly, the invention relates to methods and compositions for the modulation, diagnosis and treatment of disorders associated with these tissues and cells. Moreover, because the gene is expressed in colon to liver metastases, in one embodiment expression is important in colon cancer and in colonic metastases to the liver. The gene is also expressed in keratinocytes, and accordingly, in one embodiment expression of the gene is relevant to the development of epithelium and other structures in which keratinocytes are precursor cells. Because the gene is also expressed in lymphoma, in one embodiment,

gene expression is relevant to this disorder. Further, because the gene is expressed in osteoblasts, in one embodiment expression is relevant to bone disorders that include defects in development of proper bone mass, and accordingly, is relevant in osteoporosis and osteopetrosis.

The 2208 protein kinase expression is shown in the tissues and cells shown in Figure 25. In addition, it is also expressed in tissues or cells disclosed hereinabove. Accordingly, the invention relates to methods and compositions for the modulation, diagnosis and treatment of disorders associated with these tissues and cells. In particular, expression is relevant in tissues in which the gene is highly expressed, such as brain, heart, testis and vein. Moreover, because the gene is expressed in colon to liver metastases, in one embodiment expression is important in colon cancer and in colonic metastases to the liver. The gene is also expressed in keratinocytes, and accordingly, in one embodiment expression of the gene is relevant to the development of epithelium and other structures in which keratinocytes are precursor cells. Because the gene is also expressed in lymphoma, in one embodiment, gene expression is relevant to this disorder. Further, because the gene is expressed in osteoblasts, in one embodiment expression is relevant to bone disorders that include defects in development of proper bone mass, and accordingly, is relevant in osteoporosis and osteopetrosis.

Disorders involving the spleen include, but are not limited to, splenomegaly, including nonspecific acute splenitis, congestive spenomegaly, and spenic infarcts; neoplasms, congenital anomalies, and rupture. Disorders associated with splenomegaly include infections, such as nonspecific splenitis, infectious mononucleosis, tuberculosis, typhoid fever, brucellosis, cytomegalovirus, syphilis, malaria, histoplasmosis, toxoplasmosis, kala-azar, trypanosomiasis, schistosomiasis, leishmaniasis, and echinococcosis; congestive states related to partial hypertension, such as cirrhosis of the liver, portal or splenic vein thrombosis, and cardiac failure; lymphohematogenous disorders, such as Hodgkin disease, non-Hodgkin lymphomas/leukemia, multiple myeloma, myeloproliferative disorders, hemolytic anemias, and thrombocytopenic purpura; immunologic-inflammatory conditions, such as rheumatoid arthritis and systemic lupus erythematosus; storage diseases such as Gaucher disease, Niemann-Pick

disease, and mucopolysaccharidoses; and other conditions, such as amyloidosis, primary neoplasms and cysts, and secondary neoplasms.

Disorders involving the lung include, but are not limited to, congenital anomalies; atelectasis; diseases of vascular origin, such as pulmonary congestion and edema, including hemodynamic pulmonary edema and edema caused by microvascular injury, adult respiratory distress syndrome (diffuse alveolar damage), pulmonary embolism, hemorrhage, and infarction, and pulmonary hypertension and vascular sclerosis; chronic obstructive pulmonary disease, such as emphysema, chronic bronchitis, bronchial asthma, and bronchiectasis; diffuse interstitial (infiltrative, restrictive) diseases, such as pneumoconioses, sarcoidosis, idiopathic pulmonary fibrosis, desquamative interstitial pneumonitis, hypersensitivity pneumonitis, pulmonary eosinophilia (pulmonary infiltration with eosinophilia), *Bronchiolitis obliterans*-organizing pneumonia, diffuse pulmonary hemorrhage syndromes, including Goodpasture syndrome, idiopathic pulmonary hemosiderosis and other hemorrhagic syndromes, pulmonary involvement in collagen vascular disorders, and pulmonary alveolar proteinosis; complications of therapies, such as drug-induced lung disease, radiation-induced lung disease, and lung transplantation; tumors, such as bronchogenic carcinoma, including paraneoplastic syndromes, bronchioloalveolar carcinoma, neuroendocrine tumors, such as bronchial carcinoid, miscellaneous tumors, and metastatic tumors; pathologies of the pleura, including inflammatory pleural effusions, noninflammatory pleural effusions, pneumothorax, and pleural tumors, including solitary fibrous tumors (pleural fibroma) and malignant mesothelioma.

Disorders involving the colon include, but are not limited to, congenital anomalies, such as atresia and stenosis, Meckel diverticulum, congenital aganglionic megacolon-Hirschsprung disease; enterocolitis, such as diarrhea and dysentery, infectious enterocolitis, including viral gastroenteritis, bacterial enterocolitis, necrotizing enterocolitis, antibiotic-associated colitis (pseudomembranous colitis), and collagenous and lymphocytic colitis, miscellaneous intestinal inflammatory disorders, including parasites and protozoa, acquired immunodeficiency syndrome, transplantation, drug-induced intestinal injury, radiation enterocolitis, neutropenic colitis (typhlitis), and diversion colitis; idiopathic inflammatory bowel disease, such as Crohn disease and

ulcerative colitis; tumors of the colon, such as non-neoplastic polyps, adenomas, familial syndromes, colorectal carcinogenesis, colorectal carcinoma, and carcinoid tumors.

Disorders involving the liver include, but are not limited to, hepatic injury; jaundice and cholestasis, such as bilirubin and bile formation; hepatic failure and cirrhosis, such as cirrhosis, portal hypertension, including ascites, portosystemic shunts, and splenomegaly; infectious disorders, such as viral hepatitis, including hepatitis A-E infection and infection by other hepatitis viruses, clinicopathologic syndromes, such as the carrier state, asymptomatic infection, acute viral hepatitis, chronic viral hepatitis, and fulminant hepatitis; autoimmune hepatitis; drug- and toxin-induced liver disease, such as alcoholic liver disease; inborn errors of metabolism and pediatric liver disease, such as hemochromatosis, Wilson disease, a_1-antitrypsin deficiency, and neonatal hepatitis; intrahepatic biliary tract disease, such as secondary biliary cirrhosis, primary biliary cirrhosis, primary sclerosing cholangitis, and anomalies of the biliary tree; circulatory disorders, such as impaired blood flow into the liver, including hepatic artery compromise and portal vein obstruction and thrombosis, impaired blood flow through the liver, including passive congestion and centrilobular necrosis and peliosis hepatis, hepatic vein outflow obstruction, including hepatic vein thrombosis (Budd-Chiari syndrome) and veno-occlusive disease; hepatic disease associated with pregnancy, such as preeclampsia and eclampsia, acute fatty liver of pregnancy, and intrehepatic cholestasis of pregnancy; hepatic complications of organ or bone marrow transplantation, such as drug toxicity after bone marrow transplantation, graft-versus-host disease and liver rejection, and nonimmunologic damage to liver allografts; tumors and tumorous conditions, such as nodular hyperplasias, adenomas, and malignant tumors, including primary carcinoma of the liver and metastatic tumors.

Disorders involving the uterus and endometrium include, but are not limited to, endometrial histology in the menstrual cycle; functional endometrial disorders, such as anovulatory cycle, inadequate luteal phase, oral contraceptives and induced endometrial changes, and menopausal and postmenopausal changes; inflammations, such as chronic endometritis; adenomyosis; endometriosis; endometrial polyps; endometrial hyperplasia; malignant tumors, such as carcinoma of the endometrium; mixed Müllerian and

mesenchymal tumors, such as malignant mixed Müllerian tumors; tumors of the myometrium, including leiomyomas, leiomyosarcomas, and endometrial stromal tumors.

Disorders involving the brain include, but are not limited to, disorders involving neurons, and disorders involving glia, such as astrocytes, oligodendrocytes, ependymal cells, and microglia; cerebral edema, raised intracranial pressure and herniation, and hydrocephalus; malformations and developmental diseases, such as neural tube defects, forebrain anomalies, posterior fossa anomalies, and syringomyelia and hydromyelia; perinatal brain injury; cerebrovascular diseases, such as those related to hypoxia, ischemia, and infarction, including hypotension, hypoperfusion, and low-flow states--global cerebral ischemia and focal cerebral ischemia--infarction from obstruction of local blood supply, intracranial hemorrhage, including intracerebral (intraparenchymal) hemorrhage, subarachnoid hemorrhage and ruptured berry aneurysms, and vascular malformations, hypertensive cerebrovascular disease, including lacunar infarcts, slit hemorrhages, and hypertensive encephalopathy; infections, such as acute meningitis, including acute pyogenic (bacterial) meningitis and acute aseptic (viral) meningitis, acute focal suppurative infections, including brain abscess, subdural empyema, and extradural abscess, chronic bacterial meningoencephalitis, including tuberculosis and mycobacterioses, neurosyphilis, and neuroborreliosis (Lyme disease), viral meningoencephalitis, including arthropod-borne (Arbo) viral encephalitis, *Herpes simplex* virus Type 1, *Herpes simplex* virus Type 2, *Varicalla-zoster* virus (*Herpes zoster*), cytomegalovirus, poliomyelitis, rabies, and human immunodeficiency virus 1, including HIV-1 meningoencephalitis (subacute encephalitis), vacuolar myelopathy, AIDS-associated myopathy, peripheral neuropathy, and AIDS in children, progressive multifocal leukoencephalopathy, subacute sclerosing panencephalitis, fungal meningoencephalitis, other infectious diseases of the nervous system; transmissible spongiform encephalopathies (prion diseases); demyelinating diseases, including multiple sclerosis, multiple sclerosis variants, acute disseminated encephalomyelitis and acute necrotizing hemorrhagic encephalomyelitis, and other diseases with demyelination; degenerative diseases, such as degenerative diseases affecting the cerebral cortex, including Alzheimer disease and Pick disease, degenerative diseases of basal ganglia and brain stem, including Parkinsonism, idiopathic Parkinson disease (paralysis agitans),

progressive supranuclear palsy, corticobasal degeneration, multiple system atrophy, including striatonigral degeneration, Shy-Drager syndrome, and olivopontocerebellar atrophy, and Huntington disease; spinocerebellar degenerations, including spinocerebellar ataxias, including Friedreich ataxia, and ataxia-telanglectasia, degenerative diseases affecting motor neurons, including amyotrophic lateral sclerosis (motor neuron disease), bulbospinal atrophy (Kennedy syndrome), and spinal muscular atrophy; inborn errors of metabolism, such as leukodystrophies, including Krabbe disease, metachromatic leukodystrophy, adrenoleukodystrophy, Pelizaeus-Merzbacher disease, and Canavan disease, mitochondrial encephalomyopathies, including Leigh disease and other mitochondrial encephalomyopathies; toxic and acquired metabolic diseases, including vitamin deficiencies such as thiamine (vitamin B_1) deficiency and vitamin B_{12} deficiency, neurologic sequelae of metabolic disturbances, including hypoglycemia, hyperglycemia, and hepatic encephatopathy, toxic disorders, including carbon monoxide, methanol, ethanol, and radiation, including combined methotrexate and radiation-induced injury; tumors, such as gliomas, including astrocytoma, including fibrillary (diffuse) astrocytoma and glioblastoma multiforme, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and brain stem glioma, oligodendroglioma, and ependymoma and related paraventricular mass lesions, neuronal tumors, poorly differentiated neoplasms, including medulloblastoma, other parenchymal tumors, including primary brain lymphoma, germ cell tumors, and pineal parenchymal tumors, meningiomas, metastatic tumors, paraneoplastic syndromes, peripheral nerve sheath tumors, including schwannoma, neurofibroma, and malignant peripheral nerve sheath tumor (malignant schwannoma), and neurocutaneous syndromes (phakomatoses), including neurofibromotosis, including Type 1 neurofibromatosis (NF1) and TYPE 2 neurofibromatosis (NF2), tuberous sclerosis, and Von Hippel-Lindau disease.

Disorders involving T-cells include, but are not limited to, cell-mediated hypersensitivity, such as delayed type hypersensitivity and T-cell-mediated cytotoxicity, and transplant rejection; autoimmune diseases, such as systemic lupus erythematosus, Sjögren syndrome, systemic sclerosis, inflammatory myopathies, mixed connective tissue disease, and polyarteritis nodosa and other vasculitides; immunologic deficiency syndromes, including but not limited to, primary immunodeficiencies, such as thymic

hypoplasia, severe combined immunodeficiency diseases, and AIDS; leukopenia; reactive (inflammatory) proliferations of white cells, including but not limited to, leukocytosis, acute nonspecific lymphadenitis, and chronic nonspecific lymphadenitis; neoplastic proliferations of white cells, including but not limited to lymphoid neoplasms, such as precursor T-cell neoplasms, such as acute lymphoblastic leukemia/lymphoma, peripheral T-cell and natural killer cell neoplasms that include peripheral T-cell lymphoma, unspecified, adult T-cell leukemia/lymphoma, mycosis fungoides and Sézary syndrome, and Hodgkin disease.

Diseases of the skin, include but are not limited to, disorders of pigmentation and melanocytes, including but not limited to, vitiligo, freckle, melasma, lentigo, nevocellular nevus, dysplastic nevi, and malignant melanoma; benign epithelial tumors, including but not limited to, seborrheic keratoses, acanthosis nigricans, fibroepithelial polyp, epithelial cyst, keratoacanthoma, and adnexal (appendage) tumors; premalignant and malignant epidermal tumors, including but not limited to, actinic keratosis, squamous cell carcinoma, basal cell carcinoma, and merkel cell carcinoma; tumors of the dermis, including but not limited to, benign fibrous histiocytoma, dermatofibrosarcoma protuberans, xanthomas, and dermal vascular tumors; tumors of cellular immigrants to the skin, including but not limited to, histiocytosis X, mycosis fungoides (cutaneous T-cell lymphoma), and mastocytosis; disorders of epidermal maturation, including but not limited to, ichthyosis; acute inflammatory dermatoses, including but not limited to, urticaria, acute eczematous dermatitis, and erythema multiforme; chronic inflammatory dermatoses, including but not limited to, psoriasis, lichen planus, and lupus erythematosus; blistering (bullous) diseases, including but not limited to, pemphigus, bullous pemphigoid, dermatitis herpetiformis, and noninflammatory blistering diseases: epidermolysis bullosa and porphyria; disorders of epidermal appendages, including but not limited to, acne vulgaris; panniculitis, including but not limited to, erythema nodosum and erythema induratum; and infection and infestation, such as verrucae, molluscum contagiosum, impetigo, superficial fungal infections, and arthropod bites, stings, and infestations.

In normal bone marrow, the myelocytic series (polymorphoneuclear cells) make up approximately 60% of the cellular elements, and the erythrocytic series, 20-30%.

Lymphocytes, monocytes, reticular cells, plasma cells and megakaryocytes together constitute 10-20%. Lymphocytes make up 5-15% of normal adult marrow. In the bone marrow, cell types are add mixed so that precursors of red blood cells (erythroblasts), macrophages (monoblasts), platelets (megakaryocytes), polymorphoneuclear leucocytes (myeloblasts), and lymphocytes (lymphoblasts) can be visible in one microscopic field. In addition, stem cells exist for the different cell lineages, as well as a precursor stem cell for the committed progenitor cells of the different lineages. The various types of cells and stages of each would be known to the person of ordinary skill in the art and are found, for example, on page 42 (Figure 2-8) of *Immunology, Imunopathology and Immunity*, Fifth Edition, Sell *et al.* Simon and Schuster (1996), incorporated by reference for its teaching of cell types found in the bone marrow. According, the invention is directed to disorders arising from these cells. These disorders include but are not limited to the following: diseases involving hematopoeitic stem cells; committed lymphoid progenitor cells; lymphoid cells including B and T-cells; committed myeloid progenitors, including monocytes, granulocytes, and megakaryocytes; and committed erythroid progenitors. These include but are not limited to the leukemias, including B-lymphoid leukemias, T-lymphoid leukemias, undifferentiated leukemias; erythroleukemia, megakaryoblastic leukemia, monocytic; [leukemias are encompassed with and without differentiation]; chronic and acute lymphoblastic leukemia, chronic and acute lymphocytic leukemia, chronic and acute myelogenous leukemia, lymphoma, myelo dysplastic syndrome, chronic and acute myeloid leukemia, myelomonocytic leukemia; chronic and acute myeloblastic leukemia, chronic and acute myelogenous leukemia, chronic and acute promyelocytic leukemia, chronic and acute myelocytic leukemia, hematologic malignancies of monocyte-macrophage lineage, such as juvenile chronic myelogenous leukemia; secondary AML, antecedent hematological disorder; refractory anemia; aplastic anemia; reactive cutaneous angioendotheliomatosis; fibrosing disorders involving altered expression in dendritic cells, disorders including systemic sclerosis, E-M syndrome, epidemic toxic oil syndrome, eosinophilic fasciitis localized forms of scleroderma, keloid, and fibrosing colonopathy; angiomatoid malignant fibrous histiocytoma; carcinoma, including primary head and neck squamous cell carcinoma; sarcoma, including kaposi's sarcoma; fibroadanoma and phyllodes tumors, including

mammary fibroadenoma; stromal tumors; phyllodes tumors, including histiocytoma; erythroblastosis; neurofibromatosis; diseases of the vascular endothelium; demyelinating, particularly in old lesions; gliosis, vasogenic edema, vascular disease, Alzheimer's and Parkinson's disease; T-cell lymphomas; B-cell lymphomas.

Disorders involving the heart, include but are not limited to, heart failure, including but not limited to, cardiac hypertrophy, left-sided heart failure, and right-sided heart failure; ischemic heart disease, including but not limited to angina pectoris, myocardial infarction, chronic ischemic heart disease, and sudden cardiac death; hypertensive heart disease, including but not limited to, systemic (left-sided) hypertensive heart disease and pulmonary (right-sided) hypertensive heart disease; valvular heart disease, including but not limited to, valvular degeneration caused by calcification, such as calcific aortic stenosis, calcification of a congenitally bicuspid aortic valve, and mitral annular calcification, and myxomatous degeneration of the mitral valve (mitral valve prolapse), rheumatic fever and rheumatic heart disease, infective endocarditis, and noninfected vegetations, such as nonbacterial thrombotic endocarditis and endocarditis of systemic lupus erythematosus (Libman-Sacks disease), carcinoid heart disease, and complications of artificial valves; myocardial disease, including but not limited to dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, and myocarditis; pericardial disease, including but not limited to, pericardial effusion and hemopericardium and pericarditis, including acute pericarditis and healed pericarditis, and rheumatoid heart disease; neoplastic heart disease, including but not limited to, primary cardiac tumors, such as myxoma, lipoma, papillary fibroelastoma, rhabdomyoma, and sarcoma, and cardiac effects of noncardiac neoplasms; congenital heart disease, including but not limited to, left-to-right shunts--late cyanosis, such as atrial septal defect, ventricular septal defect, patent ductus arteriosus, and atrioventricular septal defect, right-to-left shunts--early cyanosis, such as tetralogy of fallot, transposition of great arteries, truncus arteriosus, tricuspid atresia, and total anomalous pulmonary venous connection, obstructive congenital anomalies, such as coarctation of aorta, pulmonary stenosis and atresia, and aortic stenosis and atresia, and disorders involving cardiac transplantation.

Disorders involving blood vessels include, but are not limited to, responses of vascular cell walls to injury, such as endothelial dysfunction and endothelial activation and intimal thickening; vascular diseases including, but not limited to, congenital anomalies, such as arteriovenous fistula, atherosclerosis, and hypertensive vascular disease, such as hypertension; inflammatory disease--the vasculitides, such as giant cell (temporal) arteritis, Takayasu arteritis, polyarteritis nodosa (classic), Kawasaki syndrome (mucocutaneous lymph node syndrome), microscopic polyanglitis (microscopic polyarteritis, hypersensitivity or leukocytoclastic anglitis), Wegener granulomatosis, thromboanglitis obliterans (Buerger disease), vasculitis associated with other disorders, and infectious arteritis; Raynaud disease; aneurysms and dissection, such as abdominal aortic aneurysms, syphilitic (luetic) aneurysms, and aortic dissection (dissecting hematoma); disorders of veins and lymphatics, such as varicose veins, thrombophlebitis and phlebothrombosis, obstruction of superior vena cava (superior vena cava syndrome), obstruction of inferior vena cava (inferior vena cava syndrome), and lymphangitis and lymphedema; tumors, including benign tumors and tumor-like conditions, such as hemangioma, lymphangioma, glomus tumor (glomangioma), vascular ectasias, and bacillary angiomatosis, and intermediate-grade (borderline low-grade malignant) tumors, such as Kaposi sarcoma and hemangloendothelioma, and malignant tumors, such as angiosarcoma and hemangiopericytoma; and pathology of therapeutic interventions in vascular disease, such as balloon angioplasty and related techniques and vascular replacement, such as coronary artery bypass graft surgery.

Disorders involving red cells include, but are not limited to, anemias, such as hemolytic anemias, including hereditary spherocytosis, hemolytic disease due to erythrocyte enzyme defects: glucose-6-phosphate dehydrogenase deficiency, sickle cell disease, thalassemia syndromes, paroxysmal nocturnal hemoglobinuria, immunohemolytic anemia, and hemolytic anemia resulting from trauma to red cells; and anemias of diminished erythropoiesis, including megaloblastic anemias, such as anemias of vitamin B12 deficiency: pernicious anemia, and anemia of folate deficiency, iron deficiency anemia, anemia of chronic disease, aplastic anemia, pure red cell aplasia, and other forms of marrow failure.

Disorders involving the thymus include developmental disorders, such as DiGeorge syndrome with thymic hypoplasia or aplasia; thymic cysts; thymic hypoplasia, which involves the appearance of lymphoid follicles within the thymus, creating thymic follicular hyperplasia; and thymomas, including germ cell tumors, lynphomas, Hodgkin disease, and carcinoids. Thymomas can include benign or encapsulated thymoma, and malignant thymoma Type I (invasive thymoma) or Type II, designated thymic carcinoma.

Disorders involving B-cells include, but are not limited to precursor B-cell neoplasms, such as lymphoblastic leukemia/lymphoma. Peripheral B-cell neoplasms include, but are not limited to, chronic lymphocytic leukemia/small lymphocytic lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, Burkitt lymphoma, plasma cell neoplasms, multiple myeloma, and related entities, lymphoplasmacytic lymphoma (Waldenstrōm macroglobulinemia), mantle cell lymphoma, marginal zone lymphoma (MALToma), and hairy cell leukemia.

Disorders involving the kidney include, but are not limited to, congenital anomalies including, but not limited to, cystic diseases of the kidney, that include but are not limited to, cystic renal dysplasia, autosomal dominant (adult) polycystic kidney disease, autosomal recessive (childhood) polycystic kidney disease, and cystic diseases of renal medulla, which include, but are not limited to, medullary sponge kidney, and nephronophthisis-uremic medullary cystic disease complex, acquired (dialysis-associated) cystic disease, such as simple cysts; glomerular diseases including pathologies of glomerular injury that include, but are not limited to, in situ immune complex deposition, that includes, but is not limited to, anti-GBM nephritis, Heymann nephritis, and antibodies against planted antigens, circulating immune complex nephritis, antibodies to glomerular cells, cell-mediated immunity in glomerulonephritis, activation of alternative complement pathway, epithelial cell injury, and pathologies involving mediators of glomerular injury including cellular and soluble mediators, acute glomerulonephritis, such as acute proliferative (poststreptococcal, postinfectious) glomerulonephritis, including but not limited to, poststreptococcal glomerulonephritis and nonstreptococcal acute glomerulonephritis, rapidly progressive (crescentic) glomerulonephritis, nephrotic syndrome, membranous glomerulonephritis (membranous nephropathy), minimal change disease (lipoid nephrosis), focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, IgA nephropathy (Berger

disease), focal proliferative and necrotizing glomerulonephritis (focal glomerulonephritis), hereditary nephritis, including but not limited to, Alport syndrome and thin membrane disease (benign familial hematuria), chronic glomerulonephritis, glomerular lesions associated with systemic disease, including but not limited to, systemic lupus erythematosus, Henoch-Schönlein purpura, bacterial endocarditis, diabetic glomerulosclerosis, amyloidosis, fibrillary and immunotactoid glomerulonephritis, and other systemic disorders; diseases affecting tubules and interstitium, including acute tubular necrosis and tubulointerstitial nephritis, including but not limited to, pyelonephritis and urinary tract infection, acute pyelonephritis, chronic pyelonephritis and reflux nephropathy, and tubulointerstitial nephritis induced by drugs and toxins, including but not limited to, acute drug-induced interstitial nephritis, analgesic abuse nephropathy, nephropathy associated with nonsteroidal anti-inflammatory drugs, and other tubulointerstitial diseases including, but not limited to, urate nephropathy, hypercalcemia and nephrocalcinosis, and multiple myeloma; diseases of blood vessels including benign nephrosclerosis, malignant hypertension and accelerated nephrosclerosis, renal artery stenosis, and thrombotic microangiopathies including, but not limited to, classic (childhood) hemolytic-uremic syndrome, adult hemolytic-uremic syndrome/thrombotic thrombocytopenic purpura, idiopathic HUS/TTP, and other vascular disorders including, but not limited to, atherosclerotic ischemic renal disease, atheroembolic renal disease, sickle cell disease nephropathy, diffuse cortical necrosis, and renal infarcts; urinary tract obstruction (obstructive uropathy); urolithiasis (renal calculi, stones); and tumors of the kidney including, but not limited to, benign tumors, such as renal papillary adenoma, renal fibroma or hamartoma (renomedullary interstitial cell tumor), angiomyolipoma, and oncocytoma, and malignant tumors, including renal cell carcinoma (hypernephroma, adenocarcinoma of kidney), which includes urothelial carcinomas of renal pelvis.

Disorders of the breast include, but are not limited to, disorders of development; inflammations, including but not limited to, acute mastitis, periductal mastitis, periductal mastitis (recurrent subareolar abscess, squamous metaplasia of lactiferous ducts), mammary duct ectasia, fat necrosis, granulomatous mastitis, and pathologies associated with silicone breast implants; fibrocystic changes; proliferative breast disease including, but not limited to, epithelial hyperplasia, sclerosing adenosis, and small duct papillomas; tumors including,

but not limited to, stromal tumors such as fibroadenoma, phyllodes tumor, and sarcomas, and epithelial tumors such as large duct papilloma; carcinoma of the breast including in situ (noninvasive) carcinoma that includes ductal carcinoma in situ (including Paget's disease) and lobular carcinoma in situ, and invasive (infiltrating) carcinoma including, but not limited to, invasive ductal carcinoma, no special type, invasive lobular carcinoma, medullary carcinoma, colloid (mucinous) carcinoma, tubular carcinoma, and invasive papillary carcinoma, and miscellaneous malignant neoplasms.

Disorders in the male breast include, but are not limited to, gynecomastia and carcinoma.

Disorders involving the testis and epididymis include, but are not limited to, congenital anomalies such as cryptorchidism, regressive changes such as atrophy, inflammations such as nonspecific epididymitis and orchitis, granulomatous (autoimmune) orchitis, and specific inflammations including, but not limited to, gonorrhea, mumps, tuberculosis, and syphilis, vascular disturbances including torsion, testicular tumors including germ cell tumors that include, but are not limited to, seminoma, spermatocytic seminoma, embryonal carcinoma, yolk sac tumor choriocarcinoma, teratoma, and mixed tumors, tumor of sex cord-gonadal stroma including, but not limited to, leydig (interstitial) cell tumors and sertoli cell tumors (androblastoma), and testicular lymphoma, and miscellaneous lesions of tunica vaginalis.

Disorders involving the prostate include, but are not limited to, inflammations, benign enlargement, for example, nodular hyperplasia (benign prostatic hypertrophy or hyperplasia), and tumors such as carcinoma.

Disorders involving the thyroid include, but are not limited to, hyperthyroidism; hypothyroidism including, but not limited to, cretinism and myxedema; thyroiditis including, but not limited to, hashimoto thyroiditis, subacute (granulomatous) thyroiditis, and subacute lymphocytic (painless) thyroiditis; Graves disease; diffuse and multinodular goiter including, but not limited to, diffuse nontoxic (simple) goiter and multinodular goiter; neoplasms of the thyroid including, but not limited to, adenomas, other benign tumors, and carcinomas, which include, but are not limited to, papillary carcinoma, follicular carcinoma, medullary carcinoma, and anaplastic carcinoma; and cogenital anomalies.

Disorders involving the skeletal muscle include tumors such as rhabdomyosarcoma.

Disorders involving the pancreas include those of the exocrine pancreas such as congenital anomalies, including but not limited to, ectopic pancreas; pancreatitis, including but not limited to, acute pancreatitis; cysts, including but not limited to, pseudocysts; tumors, including but not limited to, cystic tumors and carcinoma of the pancreas; and disorders of the endocrine pancreas such as, diabetes mellitus; islet cell tumors, including but not limited to, insulinomas, gastrinomas, and other rare islet cell tumors.

Disorders involving the small intestine include the malabsorption syndromes such as, celiac sprue, tropical sprue (postinfectious sprue), whipple disease, disaccharidase (lactase) deficiency, abetalipoproteinemia, and tumors of the small intestine including adenomas and adenocarcinoma.

Disorders related to reduced platelet number, thrombocytopenia, include idiopathic thrombocytopenic purpura, including acute idiopathic thrombocytopenic purpura, drug-induced thrombocytopenia, HIV-associated thrombocytopenia, and thrombotic microangiopathies: thrombotic thrombocytopenic purpura and hemolytic-uremic syndrome.

Disorders involving precursor T-cell neoplasms include precursor T lymphoblastic leukemia/lymphoma. Disorders involving peripheral T-cell and natural killer cell neoplasms include T-cell chronic lymphocytic leukemia, large granular lymphocytic leukemia, mycosis fungoides and Sézary syndrome, peripheral T-cell lymphoma, unspecified, angioimmunoblastic T-cell lymphoma, angiocentric lymphoma (NK/T-cell lymphoma[4a]), intestinal T-cell lymphoma, adult T-cell leukemia/lymphoma, and anaplastic large cell lymphoma.

Disorders involving the ovary include, for example, polycystic ovarian disease, Stein-leventhal syndrome, Pseudomyxoma peritonei and stromal hyperthecosis; ovarian tumors such as, tumors of coelomic epithelium, serous tumors, mucinous tumors, endometeriod tumors, clear cell adenocarcinoma, cystadenofibroma, brenner tumor, surface epithelial tumors; germ cell tumors such as mature (benign) teratomas, monodermal teratomas, immature malignant teratomas, dysgerminoma, endodermal sinus tumor, choriocarcinoma; sex cord-stomal tumors such as, granulosa-theca cell tumors, thecoma-fibromas, androblastomas, hill cell tumors, and gonadoblastoma; and metastatic tumors such as Krukenberg tumors.

Bone-forming cells include the osteoprogenitor cells, osteoblasts, and osteocytes. The disorders of the bone are complex because they may have an impact on the skeleton during any of its stages of development. Hence, the disorders may have variable manifestations and may involve one, multiple or all bones of the body. Such disorders include, congenital malformations, achondroplasia and thanatophoric dwarfism, diseases associated with abnormal matix such as type 1 collagen disease, osteoporosis, Paget disease, rickets, osteomalacia, high-turnover osteodystrophy, low-turnover of aplastic disease, osteonecrosis, pyogenic osteomyelitis, tuberculous osteomyelitism, osteoma, osteoid osteoma, osteoblastoma, osteosarcoma, osteochondroma, chondromas, chondroblastoma, chondromyxoid fibroma, chondrosarcoma, fibrous cortical defects, fibrous dysplasia, fibrosarcoma, malignant fibrous histiocytoma, Ewing sarcoma, primitive neuroectodermal tumor, giant cell tumor, and metastatic tumors.

Novel Kinase Sequence

The present invention is based, at least in part, on the identification of novel protein kinases and nucleic acid molecules encoding them, that comprise a family of molecules having certain conserved structural and functional features. The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence identity as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin, as well as other, distinct proteins of human origin or alternatively, can contain homologs of non-human origin. Members of a family may also have common functional characteristics.

The invention features a protein kinase nucleic acid molecule, preferably a human protein kinase molecule, identified based on a consensus motif or protein domain characteristic of the protein kinase family of proteins.

The kinase genes of the invention were identified in human cDNA libraries. The clones to which the present invention are directed contain a mRNA transcript having a corresponding cDNA sequence set forth in SEQ ID NOS:1, 3, 4, 6, 7, 9, 10, 12, 13, 15,

16, or 18. This transcript has a nucleotide open reading frame encoding an amino acid sequence set forth in SEQ ID NOS: 2, 5, 8, 11, 14 or 17, respectively.

Preferred kinase polypeptides of the present invention have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NOS:2, 5, 8, 11, 14 or 17 or a domain thereof. The term "sufficiently identical" is used herein to refer to a first amino acid or nucleotide sequence that contains a sufficient or minimum number of identical or equivalent (e.g., with a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have a common structural domain and/or common functional activity. For example, amino acid or nucleotide sequences that contain a common structural domain having at least about 45%, 55%, or 65% identity, preferably about 75% identity, more preferably about 85%, 90%, 95%, or more identity are defined herein as sufficiently identical.

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., percent identity = number of identical positions/total number of positions (e.g., overlapping positions) x 100). In one embodiment, the two sequences are the same length. The percent identity between two sequences can be determined using techniques similar to those described below, with or without allowing gaps. In calculating percent identity, typically exact matches are counted.

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (1970) *J. Mol. Biol. 48*:444-453 algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of

1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a sequence identity or homology limitation of the invention) is using a Blossum 62 scoring matrix with a gap open penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to kinase nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to kinase protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-Blast can be used to perform an iterated search that detects distant relationships between molecules. *See* Altschul *et al.* (1997) *supra.* When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. *See* http://www.ncbi.nlm.nih.gov. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0), which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

Accordingly, another embodiment of the invention features isolated kinase proteins and polypeptides having a kinase protein activity. As used interchangeably herein, a "kinase protein activity", "biological activity of a kinase protein", or "functional activity of a kinase protein" refers to an activity exerted by a kinase protein, polypeptide,

or nucleic acid molecule on a kinase-responsive cell as determined *in vivo*, or *in vitro*, according to standard assay techniques. A kinase activity can be a direct activity, such as autophosphorylation or an association with or an enzymatic activity on a second protein, such as on c-fos, c-jun, APF2, and ETS family members Elkl, Sapla, and c-Ets, or other transcription factors, targets of Cdk function or interaction, such as cyclin, the small inhibitory molecules discussed in Dynlacht, above, and Nigg, ((1995) *Bioessays 17*: 471-480), transcriptional co-activators such as p110Rb and p107 and transcription factors such as p53, E2F, and RNA polymerase II, cytoskeletal proteins and cytoplasmic signaling proteins as cited in Brott *et al.*, above, TCF/LEF factors, POP-1 and other HMG-domain-containing proteins, or an indirect activity, such as a cellular signaling activity mediated by the Wnt or MAPK pathways, for example, as discussed hereinabove. In a preferred embodiment, a kinase activity includes at least one or more of the following activities: (1) modulating (stimulating and/or enhancing or inhibiting) cellular proliferation, growth and/or metabolism (e.g. in those cells in which the sequence is expressed, including virus infected cells); (2) the regulation of transmission of signals from cellular receptors, e.g., growth factor receptors; (3) the modulation of the entry of cells into mitosis; (4) the modulation of cellular differentiation; (5) the modulation of cell death; and (6) the regulation of cytoskeleton function, e.g., actin bundling. Functions also include, but are not limited to, those shown for the specific functional sites in Figures 7-12 and 35-40, including ATP binding and phosphotransferase activity.

An "isolated" or "purified" kinase nucleic acid molecule or protein, or biologically active portion thereof, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. Preferably, an "isolated" nucleic acid is free of sequences (preferably protein-encoding sequences) that naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For purposes of the invention, "isolated" when used to refer to nucleic acid molecules excludes isolated chromosomes. For example, in various embodiments, the isolated kinase nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the nucleic acid molecule in

genomic DNA of the cell from which the nucleic acid is derived. A kinase protein that is substantially free of cellular material includes preparations of kinase protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of non-kinase protein (also referred to herein as a "contaminating protein"). When the kinase protein or biologically active portion thereof is recombinantly produced, preferably, culture medium represents less than about 30%, 20%, 10%, or 5% of the volume of the protein preparation. When kinase protein is produced by chemical synthesis, preferably the protein preparations have less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or non-kinase chemicals.

Various aspects of the invention are described in further detail in the following subsections.

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to an isolated nucleic acid molecule comprising a nucleotide sequence encoding a kinase protein or biologically active portion thereof, as well as a nucleic acid molecule sufficient for use as a hybridization probe to identify kinase-encoding nucleic acids (e.g., kinase mRNA) and fragments for use as PCR primers for the amplification or mutation of kinase nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

A nucleotide sequence encoding the kinase proteins of the present invention includes the sequence set forth in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, 12, 13, 15, 16, or 18 and the complement thereof. By "complement" is intended a nucleotide sequence that is sufficiently complementary to a given nucleotide sequence such that it can hybridize to the given nucleotide sequence to thereby form a stable duplex. The corresponding amino acid sequence for the kinase protein encoded by the nucleotide sequence is set forth in SEQ ID NO: 2, 5, 8, 11, 14, or 17.

Nucleic acid molecules that are fragments of the kinase nucleotide sequence are also encompassed by the present invention. By "fragment" is intended a portion of the

nucleotide sequence encoding a kinase protein of the invention. A fragment of a kinase nucleotide sequence may encode a biologically active portion of a kinase protein, or it may be a fragment that can be used as a hybridization probe or PCR primer using methods disclosed below. A biologically active portion of a kinase protein can be prepared by isolating a portion of the kinase nucleotide sequence of the invention, expressing the encoded portion of the kinase protein (e.g., by recombinant expression *in vitro*), and assessing the activity of the encoded portion of the kinase protein.

Generally, nucleic acid molecules that are fragments of a kinase nucleotide sequence comprise at least 15, 20, 50, 75, 100, 325, 350, 375, 400, 425, 450, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2800, 3000, 3200, 3400, 3600, 3800, 4000, 4200, 4400, 4600, 4800, 5000, 5200, 5400 nucleotides, or up to the number of nucleotides present in a full-length kinase nucleotide sequence disclosed herein depending upon the intended use.

Alternatively, a nucleic acid molecules that is a fragment of a 18477 nucleotide sequence of the present invention comprises a nucleotide sequence consisting of nucleotides 1-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300, 1300-1400, 1400-1500, 1500-1600, 1600-1700, 1700-1800, 1800-1900, 2000-2100, 2100-2200, 2200-2300, 2300-2400, 2400-2500, 2500-2600, 2600-2700, 2700-2900, 2900-3100, or 3100-3003 of SEQ ID NO:1 or 3.

A nucleic acid molecules that is a fragment of an 3695 nucleotide sequence of the present invention comprises a nucleotide sequence consisting of nucleotides 1-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300, 1300-1400, 1400-1500, 1500-1600, 1600-1700, 1700-1800, 1800-1900, 2000-2100, 2100-2200, 2200-2300, 2300-2400, 2400-2500, 2500-2600, 2600-2700, 2700-2900, 2900-3100, 3100-3300, 3300-3500, 3500-3700, 3700-3900, 3900-4100, 4100-4300, 4300-4500, 4500-4700, 4700-4900, 4900-5100, 5100-5300, 5300-5500, 5500-5700, or 5700-5983 of SEQ ID NO:4 or 6.

Alternatively, a nucleic acid molecules that is a fragment of an 13302 nucleotide sequence of the present invention comprises a nucleotide sequence consisting of nucleotides 1-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800,

800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300, 1300-1400, 1400-1500, 1500-1600, 1600-1700, 1700-1800, 1800-1900, 2000-2100, 2100-2200, or 2200-2389 of SEQ ID NO:7 or 9.

Alternatively, a nucleic acid molecules that is a fragment of an 2208 nucleotide sequence of the present invention comprises a nucleotide sequence consisting of nucleotides 1-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300, 1300-1400, 1400-1500, 1500-1600, 1600-1700, 1700-1800, 1800-1900, 1900-2100, or 2100-2162 of SEQ ID NO:10 or 12.

Alternatively, a nucleic acid molecules that is a fragment of an 2193 nucleotide sequence of the present invention comprises a nucleotide sequence consisting of nucleotides 1-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300, 1300-1400, 1400-1500, 1500-1600, 1600-1700, or 1700-1826 of SEQ ID NO:13 or 15.

Alternatively, a nucleic acid molecules that is a fragment of an 2249 nucleotide sequence of the present invention comprises a nucleotide sequence consisting of nucleotides 1-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300, 1300-1400, 1400-1500, 1500-1600, 1600-1700, 1700-1800, 1800-1900, 1900-2100, 2100-2200, 2200-2300, 2300-2400, 2400-2500, 2500-2600, 2600-2700, or 2700-2870 of SEQ ID NO:16 or 18.

It is understood that isolated fragments include any contiguous sequence not disclosed prior to the invention as well as sequences that are substantially the same and which are not disclosed. Accordingly, if a fragment is disclosed prior to the present invention, that fragment is not intended to be encompassed by the invention. When a sequence is not disclosed prior to the present invention, an isolated nucleic acid fragment is at least about 12, 15, 20, 25, or 30 contiguous nucleotides. Other regions of the nucleotide sequence may comprise fragments of various sizes, depending upon potential homology with previously disclosed sequences. Further, the sizes of the fragments may vary depending on the region analyzed.

Generally, a fragment of a kinase nucleotide sequence that encodes a biologically active portion of a kinase protein of the invention will encode at least 15, 25, 30, 50, 75,

100, 125, 150, 160, or 170 contiguous amino acids, or up to the total number of amino acids present in a full-length kinase protein of the invention. Fragments of a kinase nucleotide sequence that are useful as hybridization probes for PCR primers generally need not encode a biologically active portion of a kinase protein.

Nucleic acid molecules that are variants of the kinase nucleotide sequence disclosed herein are also encompassed by the present invention. "Variants" of the kinase nucleotide sequence include those sequences that encode the kinase protein disclosed herein but that differ conservatively because of the degeneracy of the genetic code. These naturally-occurring allelic variants can be identified with the use of well-known molecular biology techniques, such as polymerase chain reaction (PCR) and hybridization techniques as outlined below. Variant nucleotide sequences also include synthetically-derived nucleotide sequences that have been generated, for example, by using site-directed mutagenesis but which still encode the kinase protein disclosed in the present invention as discussed below. Generally, nucleotide sequence variants of the invention will have at least 45%, 55%, 65%, 75%, 85%, 95%, or 98% identity to the nucleotide sequence disclosed herein. A variant kinase nucleotide sequence will encode a kinase protein that has the amino acid sequence having at least 45%, 55%, 65%, 75%, 85%, 95%, or 98% identity to the amino acid sequence of the kinase protein disclosed herein.

In addition to the kinase nucleotide sequences shown in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, 12, 13, 15, 16, or 18, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the kinase protein may exist within a population (e.g., the human population). Such genetic polymorphism in a kinase gene may exist among individuals within a population due to natural allelic variation. An allele is one of a group of genes that occur alternatively at a given genetic locus. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a kinase protein, preferably a mammalian kinase protein. As used herein, the phrase "allelic variant" refers to a nucleotide sequence that occurs at a kinase locus or to a polypeptide encoded by the nucleotide sequence. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the kinase gene. Any and all such nucleotide variations and

resulting amino acid polymorphisms or variations in a kinase sequence that are the result of natural allelic variation and that do not alter the functional activity of kinase proteins are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding kinase protein from other species (kinase homologs), which have a nucleotide sequence differing from that of the kinase sequence disclosed herein, are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologs of the kinase cDNA of the invention can be isolated based on their identity to the kinase nucleic acid disclosed herein using the cDNA, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions as disclosed below.

In addition to naturally-occurring allelic variants of the kinase sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of the invention thereby leading to changes in the amino acid sequence of the encoded kinase protein, without altering the biological activity of the kinase protein. Thus, an isolated nucleic acid molecule encoding a kinase protein having a sequence that differs from those of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, 12, 13, 15, 16, or 18 can be created by introducing one or more nucleotide substitutions, additions, or deletions into the nucleotide sequences disclosed herein, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Such variant nucleotide sequences are also encompassed by the present invention.

For example, preferably, conservative amino acid substitutions may be made at one or more predicted, preferably nonessential amino acid residues. A "nonessential" amino acid residue is a residue that can be altered from the wild-type sequence of a kinase protein (e.g., a sequence of SEQ ID NO:2, 5, 8, 11, 14, or 17) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families

include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Such substitutions would not be made for conserved amino acid residues or for amino acid residues residing within a conserved protein domain, such as the serine/threonine protein kinase domain of the disclosed clones, where such residues are essential for protein activity.

Alternatively, variant kinase nucleotide sequences can be made by introducing mutations randomly along all or part of the kinase coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for kinase biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed recombinantly, and the activity of the protein can be determined using standard assay techniques.

Thus the nucleotide sequences of the invention include the sequences disclosed herein as well as fragments and variants thereof. The kinase nucleotide sequences of the invention, and fragments and variants thereof, can be used as probes and/or primers to identify and/or clone kinase homologs in other cell types, e.g., from other tissues, as well as kinase homologs from other mammals. Such probes can be used to detect transcripts or genomic sequences encoding the same or identical proteins. These probes can be used as part of a diagnostic test kit for identifying cells or tissues that misexpress a kinase protein, such as by measuring levels of a kinase-encoding nucleic acid in a sample of cells from a subject, e.g., detecting kinase mRNA levels or determining whether a genomic kinase gene has been mutated or deleted.

In this manner, methods such as PCR, hybridization, and the like can be used to identify such sequences having substantial identity to the sequences of the invention. *See*, for example, Sambrook *et al.* (1989) *Molecular Cloning: Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, NY) and Innis, *et al.* (1990) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, NY). Kinase nucleotide sequences isolated based on their sequence identity to the kinase nucleotide

sequences set forth herein or to fragments and variants thereof are encompassed by the present invention.

In a hybridization method, all or part of a known kinase nucleotide sequence can be used to screen cDNA or genomic libraries. Methods for construction of such cDNA and genomic libraries are generally known in the art and are disclosed in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, NY). The so-called hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labeled with a detectable group such as ^{32}P, or any other detectable marker, such as other radioisotopes, a fluorescent compound, an enzyme, or an enzyme co-factor. Probes for hybridization can be made by labeling synthetic oligonucleotides based on the known kinase nucleotide sequences disclosed herein. Degenerate primers designed on the basis of conserved nucleotides or amino acid residues in a known kinase nucleotide sequence or encoded amino acid sequence can additionally be used. The probe typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, preferably about 25, more preferably about 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, or 400 consecutive nucleotides of a kinase nucleotide sequence of the invention or a fragment or variant thereof. Preparation of probes for hybridization is generally known in the art and is disclosed in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York), herein incorporated by reference.

For example, in one embodiment, a previously unidentified kinase nucleic acid molecule hybridizes under stringent conditions to a probe that is a nucleic acid molecule comprising a kinase nucleotide sequence of the invention or a fragment thereof. In another embodiment, the previously unknown kinase nucleic acid molecule is at least 300, 325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 2,000, 3,000, or 4,000 nucleotides in length and hybridizes under stringent conditions to a probe that is a nucleic acid molecule comprising a kinase nucleotide sequence disclosed herein or a fragment thereof.

Accordingly, in another embodiment, an isolated previously unknown kinase nucleic acid molecule of the invention is at least 300, 325, 350, 375, 400, 425, 450, 500,

518, 550, 600, 650, 700, 800, 831, 900, 981, 1000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, or 2,060 nucleotides in length and hybridizes under stringent conditions to a probe that is a nucleic acid molecule comprising one of the nucleotide sequences of the invention, preferably a coding sequence set forth in SEQ ID NO:1, 3, 4, 6, 7, 9, 10, 12, 13, 15, 16, 18 or a complement, fragment, or variant thereof.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology* (John Wiley & Sons, New York (1989)), 6.3.1-6.3.6. A preferred, example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50°C. Another example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 55°C. A further example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C. Preferably, stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C. Particularly preferred stringency conditions (and the conditions that should be used if the practitioner is uncertain about what conditions should be applied to determine if a molecule is within a hybridization limitation of the invention) are 0.5M Sodium Phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Preferably, an isolated nucleic acid molecule that hybridizes under stringent conditions to an kinase sequence of the invention corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

Thus, in addition to the kinase nucleotide sequences disclosed herein and fragments and variants thereof, the isolated nucleic acid molecules of the invention also encompasses homologous DNA sequences identified and isolated from other cells and/or

organisms by hybridization with entire or partial sequences obtained from a kinase nucleotide sequence disclosed herein or variants and fragments thereof.

The present invention also encompasses antisense nucleic acid molecules, i.e., molecules that are complementary to a sense nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule, or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire kinase coding strand, or to only a portion thereof, e.g., all or part of the protein coding region (or open reading frame). An antisense nucleic acid molecule can be antisense to a noncoding region of the coding strand of a nucleotide sequence encoding a kinase protein. The noncoding regions are the 5' and 3' sequences that flank the coding region and are not translated into amino acids.

Given the coding-strand sequence encoding a kinase protein disclosed herein (e.g., SEQ ID NOS:1, 3, 4, 6, 7, 9, 10, 12, 13, 15, 16, or 18), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of kinase mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of kinase mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of kinase mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation procedures known in the art.

For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally-occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, including, but not limited to, for example, phosphorothioate derivatives and acridine substituted nucleotides. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in

an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a kinase protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, antisense molecules can be linked to peptides or antibodies to form a complex that specifically binds to receptors or antigens expressed on a selected cell surface. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

An antisense nucleic acid molecule of the invention can be an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

The invention also encompasses ribozymes, which are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave kinase mRNA transcripts to thereby inhibit translation of kinase mRNA. A ribozyme having specificity for a kinase-encoding nucleic acid can be designed based upon the nucleotide sequence of a kinase cDNA disclosed herein (e.g., SEQ ID NOS:1, 3, 4, 6, 7, 9, 10, 12, 13, 15, 16, or 18). *See,* e.g., Cech *et al.*, U.S. Patent No. 4,987,071; and Cech *et al.*, U.S. Patent No. 5,116,742.

Alternatively, kinase mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. *See, e.g.*, Bartel and Szostak (1993) *Science* 261:1411-1418.

The invention also encompasses nucleic acid molecules that form triple helical structures. For example, kinase gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the kinase protein (e.g., the kinase promoter and/or enhancers) to form triple helical structures that prevent transcription of the kinase gene in target cells. *See generally* Helene (1991) *Anticancer Drug Des.* 6(6):569; Helene (1992) *Ann. N.Y. Acad. Sci.* 660:27; and Maher (1992) *Bioassays* 14(12):807.

In preferred embodiments, the nucleic acid molecules of the invention can be modified at the base moiety, sugar moiety, or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (*see* Hyrup *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4:5). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid-phase peptide synthesis protocols as described in Hyrup *et al.* (1996), *supra*; Perry-O'Keefe *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93:14670.

PNAs of a kinase molecule can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigen agents for sequence-specific modulation of gene expression by, e.g., inducing transcription or translation arrest or inhibiting replication. PNAs of the invention can also be used, e.g., in the analysis of single base pair mutations in a gene by, e.g., PNA-directed PCR clamping, as artificial restriction enzymes when used in combination with other enzymes, e.g., S1 nucleases (Hyrup (1996), *supra,* or as probes or primers for DNA sequence and hybridization (Hyrup (1996), *supra*; Perry-O'Keefe *et al.* (1996), *supra*).

In another embodiment, PNAs of a kinase molecule can be modified, e.g., to enhance their stability, specificity, or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996), *supra;* Finn *et al.* (1996) *Nucleic Acids Res.* 24(17):3357-63; Mag *et al.* (1989) *Nucleic Acids Res.* 17:5973; and Peterser *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5:1119.

II. Isolated Kinase Proteins and Anti-kinase Antibodies

Kinase proteins are also encompassed within the present invention. By "kinase protein" is intended a protein having an amino acid sequence set forth in SEQ ID NO: 2, 5, 8, 11, 14, or 17 as well as fragments, biologically active portions, and variants thereof.

"Fragments" or "biologically active portions" include polypeptide fragments suitable for use as immunogens to raise anti-kinase antibodies. Fragments include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequences of a kinase protein of the invention and exhibiting at least one activity of a kinase protein, but which include fewer amino acids than the full-length kinase protein disclosed herein. Typically, biologically active portions comprise a domain or motif with at least one activity of the kinase protein. A biologically active portion of a kinase protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length. Such biologically active portions can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native kinase protein.

Alternatively, a fragment of a polypeptide of the present invention comprises an amino acid sequence consisting of amino acid residues 1-20, 20-40, 40-60, 60-80, 80-100, 100-120, 120-140, 140-160, 160-180, 180-200, 200-220, 220-240, 240-260, 260-280, 280-300, 300-320, 320-340, 340-360, 360-380, 380-400, 400-420, 420-440, 440-460, 460-480, 480-520, 520-560, 560-600, 600-640, 640-680, 680-720, 720-760, 760-800, 800-840, or 840-880 of SEQ ID NO:2.

Alternatively, a fragment of a polypeptide of the present invention comprises an amino acid sequence consisting of amino acid residues 1-20, 20-40, 40-60, 60-80, 80-

100, 100-120, 120-140, 140-160, 160-180, 180-200, 200-220, 220-240, 240-260, 260-280, 280-300, 300-320, 320-340, 340-360, 360-380, 380-400, 400-420, 420-440, 440-460, 460-480, 480-520, 520-560, 560-600, 600-640, 640-680, 680-720, 720-760, 760-800, 800-840, 840-880, 880-920, 920-960, 960-1000, 1000-1040, 1040-1080, 1080-1120, 1120-1160, 1160-1204 of SEQ ID NO:5.

Alternatively, a fragment of a polypeptide of the present invention comprises an amino acid sequence consisting of amino acid residues 1-20, 20-40, 40-60, 60-80, 80-100, 100-120, 120-140, 140-160, 160-180, 180-200, 200-220, 220-240, 240-260, 260-280, 280-300, 300-320, 320-340, 340-359 of SEQ ID NO:8.

Alternatively, a fragment of a polypeptide of the present invention comprises an amino acid sequence consisting of amino acid residues 1-20, 20-40, 40-60, 60-80, 80-100, 100-120, 120-140, 140-160, 160-180, 180-200, 200-220, 220-240, 240-260, 260-280, 280-300, 300-320, 320-340, 340-360, 360-380, 380-400, 400-420, 420-440, 440-460, 460-480, 480-520, 520-560, 560-581 of SEQ ID NO:11.

Alternatively, a fragment of a polypeptide of the present invention comprises an amino acid sequence consisting of amino acid residues 1-20, 20-40, 40-60, 60-80, 80-100, 100-120, 120-140, 140-160, 160-180, 180-200, 200-220, 220-240, 240-260, 260-280, 280-300, 300-320, 320-340, 340-360, 360-380, 380-400, 400-419 of SEQ ID NO:14.

Alternatively, a fragment of a polypeptide of the present invention comprises an amino acid sequence consisting of amino acid residues 1-20, 20-40, 40-60, 60-80, 80-100, 100-120, 120-140, 140-160, 160-180, 180-200, 200-220, 220-240, 240-260, 260-280, 280-300, 300-320, 320-340, 340-360, 360-380, 380-400, 400-420, 420-440, 440-460, 460-480, 480-520, 520-560, 560-600, and 600-629 of SEQ ID NO:17.

By "variants" is intended proteins or polypeptides having an amino acid sequence that is at least about 45%, 55%, 65%, preferably about 75%, 85%, 95%, or 98% identical to the amino acid sequence of SEQ ID NO:2, 5, 8, 11, 14, or 17. Variants also include polypeptides encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number PTA-1775, PTA-2204, PTA-1868, ______, ______, ______, or polypeptides encoded by a nucleic acid molecule that hybridizes to the nucleic acid molecule of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, 12, 13, 15, 16 or 18, or a complement

thereof, under stringent conditions. In another embodiment, a variant of an isolated polypeptide of the present invention differs, by at least 1, but less than 5, 10, 20, 50, or 100 amino acid residues from the sequence shown in SEQ ID NO:2, 5, 8, 11, 14, or 17. If alignment is needed for this comparison the sequences should be aligned for maximum identity. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences. Such variants generally retain the functional activity of the kinase-like proteins of the invention. Variants include polypeptides that differ in amino acid sequence due to natural allelic variation or mutagenesis.

The invention also provides kinase chimeric or fusion proteins. As used herein, a kinase "chimeric protein" or "fusion protein" comprises a kinase polypeptide operably linked to a non-kinase polypeptide. A "kinase polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a kinase protein, whereas a "non-kinase polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially identical to the kinase protein, e.g., a protein that is different from the kinase protein and which is derived from the same or a different organism. Within a kinase fusion protein, the kinase polypeptide can correspond to all or a portion of a kinase protein, preferably at least one biologically active portion of a kinase protein. Within the fusion protein, the term "operably linked" is intended to indicate that the kinase polypeptide and the non-kinase polypeptide are fused in-frame to each other. The non-kinase polypeptide can be fused to the N-terminus or C-terminus of the kinase polypeptide.

One useful fusion protein is a GST-kinase fusion protein in which the kinase sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant kinase proteins.

In yet another embodiment, the fusion protein is a kinase-immunoglobulin fusion protein in which all or part of a kinase protein is fused to sequences derived from a member of the immunoglobulin protein family. The kinase-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a kinase ligand and a kinase protein on the surface of a cell, thereby suppressing kinase-mediated signal transduction *in vivo*. The kinase-immunoglobulin fusion proteins can be used to affect the

bioavailability of a kinase cognate ligand. Inhibition of the kinase ligand/kinase interaction may be useful therapeutically, both for treating proliferative and differentiative disorders and for modulating (e.g., promoting or inhibiting) cell survival. Moreover, the kinase-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-kinase antibodies in a subject, to purify kinase ligands, and in screening assays to identify molecules that inhibit the interaction of a kinase protein with a kinase ligand.

Preferably, a kinase chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences may be ligated together in-frame, or the fusion gene can be synthesized, such as with automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments, which can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see*, e.g., Ausubel *et al.*, eds. (1995) *Current Protocols in Molecular Biology*) (Greene Publishing and Wiley-Interscience, NY). Moreover, a kinase-encoding nucleic acid can be cloned into a commercially available expression vector such that it is linked in-frame to an existing fusion moiety. Variants of the kinase proteins can function as either kinase agonists (mimetics) or as kinase antagonists. Variants of the kinase protein can be generated by mutagenesis, e.g., discrete point mutation or truncation of the kinase protein. An agonist of the kinase protein can retain substantially the same or a subset of the biological activities of the naturally-occurring form of the kinase protein. An antagonist of the kinase protein can inhibit one or more of the activities of the naturally-occurring form of the kinase protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade that includes the kinase protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Treatment of a subject with a variant having a subset of the biological activities of the naturally-occurring form of the protein can have fewer side effects in a subject relative to treatment with the naturally occurring form of the kinase proteins.

Variants of the kinase protein that function as either kinase agonists or as kinase antagonists can be identified by screening combinatorial libraries of mutants, e.g.,

truncation mutants, of the kinase protein for kinase protein agonist or antagonist activity. In one embodiment, a variegated library of kinase variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of kinase variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential kinase sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of kinase sequences therein. There are a variety of methods that can be used to produce libraries of potential kinase variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential kinase sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (*see*, e.g., Narang (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477).

In addition, libraries of fragments of the kinase protein coding sequence can be used to generate a variegated population of kinase fragments for screening and subsequent selection of variants of a kinase protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double-stranded PCR fragment of a kinase coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double-stranded DNA, renaturing the DNA to form double-stranded DNA which can include sense/antisense pairs from different nicked products, removing single-stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, one can derive an expression library that encodes N-terminal and internal fragments of various sizes of the kinase protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of

kinase proteins. The most widely used techniques, which are amenable to high through-put analysis for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify kinase variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

An isolated kinase polypeptide of the invention can be used as an immunogen to generate antibodies that bind kinase proteins using standard techniques for polyclonal and monoclonal antibody preparation. The full-length kinase protein can be used or, alternatively, the invention provides antigenic peptide fragments of the kinase protein for use as immunogens. The antigenic peptide of the kinase protein comprises at least 8, preferably 10, 15, 20, or 30 amino acid residues of an amino acid sequence shown in SEQ ID NOS:2, 5, 8, 11, 14, and 17, and encompasses an epitope of a kinase protein such that an antibody raised against the peptide forms a specific immune complex with the kinase protein. Preferred epitopes encompassed by the antigenic peptide are regions of a kinase protein that are located on the surface of the protein, e.g., hydrophilic regions.

Accordingly, another aspect of the invention pertains to anti-kinase polyclonal and monoclonal antibodies that bind a kinase protein. Polyclonal anti-kinase antibodies can be prepared by immunizing a suitable subject (e.g., rabbit, goat, mouse, or other mammal) with a kinase immunogen. The anti-kinase antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized kinase protein. At an appropriate time after immunization, e.g., when the anti-kinase antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497, the human B-cell hybridoma technique (Kozbor *et al.* (1983) *Immunol. Today* 4:72), the EBV-

hybridoma technique (Cole *et al.* (1985) in *Monoclonal Antibodies and Cancer Therapy*, ed. Reisfeld and Sell (Alan R. Liss, Inc., New York, NY), pp. 77-96) or trioma techniques. The technology for producing hybridomas is well known (*see* generally Coligan *et al.*, eds. (1994) *Current Protocols in Immunology* (John Wiley & Sons, Inc., New York, NY); Galfre *et al.* (1977) *Nature* 266:55052; Kenneth (1980) in *Monoclonal Antibodies: A New Dimension In Biological Analyses* (Plenum Publishing Corp., NY; and Lerner (1981) *Yale J. Biol. Med.*, 54:387-402).

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-kinase antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with a kinase protein to thereby isolate immunoglobulin library members that bind the kinase protein. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication Nos. WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; 93/01288; WO 92/01047; 92/09690; and 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J.* 12:725-734.

Additionally, recombinant anti-kinase antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and nonhuman portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT Publication Nos. WO 86101533 and WO 87/02671; European Patent Application Nos. 184,187, 171,496, 125,023, and 173,494; U.S. Patent Nos. 4,816,567 and 5,225,539; European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et*

al. (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559); Morrison (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *Bio/Techniques* 4:214; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Such antibodies can be produced using transgenic mice that are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. *See*, for example, Lonberg and Huszar (1995) *Int. Rev. Immunol.* 13:65-93); and U.S. Patent Nos. 5,625,126; 5,633,425; 5,569,825; 5,661,016; and 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, CA), can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies that recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a murine antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. This technology is described by Jespers *et al.* (1994) *Bio/Technology* 12:899-903).

An anti-kinase antibody (e.g., monoclonal antibody) can be used to isolate kinase proteins by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-kinase antibody can facilitate the purification of natural kinase protein from cells and of recombinantly produced kinase protein expressed in host cells. Moreover, an anti-kinase antibody can be used to detect kinase protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the kinase protein. Anti-kinase antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include

streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S, or ^{3}H.

Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine). The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, .alpha.-interferon, .beta.-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophase colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84:Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982). Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a kinase protein (or a portion thereof). "Vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked, such as a "plasmid", a circular double-stranded DNA loop into which additional DNA segments can be ligated, or a viral vector, where additional DNA segments can be ligated into the viral genome. The vectors are useful for autonomous replication in a host cell or may be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome (e.g., nonepisomal mammalian vectors). Expression vectors are capable of directing the expression of genes to which they are operably linked. In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids (vectors). However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication-defective retroviruses, adenoviruses, and adeno-associated viruses), that serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell. This means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, operably linked to the nucleic acid sequence to be expressed. "Operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers, and other expression control elements (e.g., polyadenylation signals). *See*, for example, Goeddel (1990) in *Gene Expression Technology: Methods in Enzymology* 185 (Academic Press, San Diego, CA). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells and those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., kinase proteins, mutant forms of kinase proteins, fusion proteins, etc.).

It is further recognized that the nucleic acid sequences of the invention can be altered to contain codons, which are preferred, or non preferred, for a particular expression system. For example, the nucleic acid can be one in which at least one altered codon, and preferably at least 10%, or 20% of the codons have been altered such that the sequence is optimized for expression in *E. coli*, yeast, human, insect, or CHO cells. Methods for determining such codon usage are well known in the art.

The recombinant expression vectors of the invention can be designed for expression of kinase protein in prokaryotic or eukaryotic host cells. Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or nonfusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein,

usually to the amino terminus of the recombinant protein. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA), and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein. Examples of suitable inducible nonfusion *E. coli* expression vectors include pTrc (Amann *et al.* (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.* (1990) in *Gene Expression Technology: Methods in Enzymology* 185 (Academic Press, San Diego, CA), pp. 60-89). Strategies to maximize recombinant protein expression in *E. coli* can be found in Gottesman (1990) in *Gene Expression Technology: Methods in Enzymology* 185 (Academic Press, CA), pp. 119-128 and Wada *et al.* (1992) *Nucleic Acids Res.* 20:2111-2118. Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter.

Suitable eukaryotic host cells include insect cells (examples of Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39)); yeast cells (examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari *et al.* (1987) *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.* (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and pPicZ (Invitrogen Corporation, San Diego, CA)); or mammalian cells (mammalian expression vectors include pCDM8 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187:195)). Suitable mammalian cells include Chinese hamster ovary cells (CHO) or COS cells. In mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus, and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells, *see* chapters 16 and 17 of Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, NY). *See*, Goeddel (1990) in *Gene Expression Technology: Methods in Enzymology* 185 (Academic Press, San Diego, CA). Alternatively, the recombinant

expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell but are still included within the scope of the term as used herein. A "purified preparation of cells", as used herein, refers to, in the case of plant or animal cells, an *in vitro* preparation of cells and not an entire intact plant or animal. In the case of cultured cells or microbial cells, it consists of a preparation of at least 10% and more preferably 50% of the subject cells.

In one embodiment, the expression vector is a recombinant mammalian expression vector that comprises tissue-specific regulatory elements that direct expression of the nucleic acid preferentially in a particular cell type. Suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), particular promoters of T-cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Patent Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379), the α-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546), and the like.

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operably linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to kinase mRNA. Regulatory sequences operably linked to a

nucleic acid cloned in the antisense orientation can be chosen to direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen to direct constitutive, tissue-specific, or cell-type-specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid, or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub *et al.* (1986) *Reviews - Trends in Genetics*, Vol. 1(1).

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook *et al.* (1989) *Molecular Cloning: A Laboraty Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, NY) and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin, and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a kinase protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) kinase protein. Accordingly, the invention

further provides methods for producing kinase protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of the invention, into which a recombinant expression vector encoding a kinase protein has been introduced, in a suitable medium such that kinase protein is produced. In another embodiment, the method further comprises isolating kinase protein from the medium or the host cell.

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which a kinase-coding sequence has been introduced. Such host cells can then be used to create nonhuman transgenic animals in which exogenous kinase sequences have been introduced into their genome or homologous recombinant animals in which endogenous kinase sequences have been altered. Such animals are useful for studying the function and/or activity of kinase genes and proteins and for identifying and/or evaluating modulators of kinase activity. As used herein, a "transgenic animal" is a nonhuman animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include nonhuman primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a nonhuman animal, preferably a mammal, more preferably a mouse, in which an endogenous kinase gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing kinase-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The kinase cDNA sequence can be introduced as a transgene into the genome of a nonhuman animal. Alternatively, a homolog of the kinase

gene can be isolated based on hybridization and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the kinase transgene to direct expression of kinase protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866, 4,870,009, and 4,873,191 and in Hogan (1986) *Manipulating the Mouse Embryo* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the kinase transgene in its genome and/or expression of kinase mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding kinase gene can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, one prepares a vector containing at least a portion of a kinase gene or a homolog of the gene into which a deletion, addition, or substitution has been introduced to thereby alter, e.g., functionally disrupt, the kinase gene. In a preferred embodiment, the vector is designed such that, upon homologous recombination, the endogenous kinase gene is functionally disrupted (i.e., no longer encodes a functional protein; such vectors are also referred to as "knock out" vectors). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous kinase gene is mutated or otherwise altered but still encodes functional protein (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous kinase protein). In the homologous recombination vector, the altered portion of the kinase gene is flanked at its 5' and 3' ends by additional nucleic acid of the kinase gene to allow for homologous recombination to occur between the exogenous kinase gene carried by the vector and an endogenous kinase gene in an embryonic stem cell. The additional flanking kinase nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (*see*, e.g., Thomas

and Capecchi (1987) *Cell* 51:503 for a description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (e.g., by electroporation), and cells in which the introduced kinase gene has homologously recombined with the endogenous kinase gene are selected (*see*, e.g., Li *et al.* (1992) *Cell* 69:915). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (*see*, e.g., Bradley (1987) in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, ed. Robertson (IRL, Oxford), pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously-recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley (1991) *Current Opinion in Bio/Technology* 2:823-829 and in PCT Publication Nos. WO 90/11354, WO 91/01140, WO 92/0968, and WO 93/04169.

In another embodiment, transgenic nonhuman animals containing selected systems that allow for regulated expression of the transgene can be produced. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, *see*, e.g., Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355). If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the nonhuman transgenic animals described herein can also be produced according to the methods described in Wilmut *et al.* (1997) *Nature* 385:810-813 and PCT Publication Nos. WO 97/07668 and WO 97/07669.

In general, methods for producing transgenic animals include introducing a nucleic acid sequence according to the present invention, the nucleic acid sequence capable of expressing the protein in a transgenic animal, into a cell in culture or *in vivo*. When introduced *in vivo*, the nucleic acid is introduced into an intact organism such that one or more cell types and, accordingly, one or more tissue types, express the nucleic acid encoding the protein. Alternatively, the nucleic acid can be introduced into virtually all cells in an organism by transfecting a cell in culture, such as an embryonic stem cell, as described herein for the production of transgenic animals, and this cell can be used to produce an entire transgenic organism. As described, in a further embodiment, the host cell can be a fertilized oocyte. Such cells are then allowed to develop in a female foster animal to produce the transgenic organism.

IV. Pharmaceutical Compositions

The kinase nucleic acid molecules, kinase proteins, and anti-kinase antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

The compositions of the invention are useful to treat any of the disorders discussed herein. The compositions are provided in therapeutically effective amounts. By "therapeutically effective amounts" is intended an amount sufficient to modulate the desired response. As defined herein, a therapeutically effective amount of protein or polypeptide (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg

body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight.

The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein, or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e.,. including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the

effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention. Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (e.g., a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes, or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF; Parsippany, NJ), or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride, in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a kinase protein or anti-kinase antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying, which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral

therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth, or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. For administration by inhalation, the compounds are delivered in the form of an aerosol spray from a pressurized container or dispenser that contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art. The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova

Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated with each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. Depending on the type and severity of the disease, about 1 μg/kg to about 15 mg/kg (e.g., 0.1 to 20 mg/kg) of antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 μg/kg to about 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays. An exemplary dosing regimen is disclosed in WO 94/04188. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (U.S. Patent 5,328,470), or by stereotactic injection (*see*, e.g., Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the

pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

V. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologs, and antibodies described herein can be used in one or more of the following methods: (a) screening assays; (b) detection assays (e.g., chromosomal mapping, tissue typing, forensic biology); (c) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenomics); and (d) methods of treatment (e.g., therapeutic and prophylactic). The isolated nucleic acid molecules of the invention can be used to express kinase protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect kinase mRNA (e.g., in a biological sample) or a genetic lesion in a kinase gene, and to modulate kinase activity. In addition, the kinase proteins can be used to screen drugs or compounds that modulate cellular growth and/or metabolism as well as to treat disorders characterized by insufficient or excessive production of kinase protein or production of kinase protein forms that have decreased or aberrant activity compared to kinase wild type protein. In addition, the anti-kinase antibodies of the invention can be used to detect and isolate kinase proteins and modulate kinase activity.

A. Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules, or other drugs) that bind to kinase proteins or have a stimulatory or inhibitory effect on, for example, kinase expression or kinase activity.

The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including biological libraries, spatially-addressable parallel solid phase or solution phase libraries,

synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, nonpeptide oligomer, or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Bio/Techniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (U.S. Patent No. 5,223,409), spores (U.S. Patent Nos. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:1865-1869), or phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 249:404-406; Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:6378-6382; and Felici (1991) *J. Mol. Biol.* 222:301-310).

Determining the ability of the test compound to bind to the kinase protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the kinase protein or biologically active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

In a similar manner, one may determine the ability of the kinase protein to bind to or interact with a kinase target molecule. By "target molecule" is intended a molecule with which a kinase protein binds or interacts in nature. In a preferred embodiment, the

ability of the kinase protein to bind to or interact with a kinase target molecule can be determined by monitoring the activity of the target molecule. For example, the activity of the target molecule can be monitored by detecting induction of a cellular second messenger of the target (e.g., intracellular Ca^{2+}, diacylglycerol, IP3, etc.), detecting catalytic/enzymatic activity of the target on an appropriate substrate, detecting the induction of a reporter gene (e.g., a kinase-responsive regulatory element operably linked to a nucleic acid encoding a detectable marker, e.g., luciferase), or detecting a cellular response, for example, viral infection, cellular differentiation or cell proliferation. Biochemical events, substrates, and effector molecules, include but are not limited to, those discussed above, including those functions shown in the figures and NLK related functions, including MAPK/Erk and Cdk-like functions.

In yet another embodiment, an assay of the present invention is a cell-free assay comprising contacting a kinase protein or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to the kinase protein or biologically active portion thereof. Binding of the test compound to the kinase protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the kinase protein or biologically active portion thereof with a known compound that binds kinase protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to preferentially bind to kinase protein or biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-free assay comprising contacting kinase protein or biologically active portion thereof with a test compound and determining the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the kinase protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of a kinase protein can be accomplished, for example, by determining the ability of the kinase protein to bind to a kinase target molecule as described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of a kinase protein can be accomplished by determining the ability of the kinase protein to further modulate

a kinase target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described.

In yet another embodiment, the cell-free assay comprises contacting the kinase protein or biologically active portion thereof with a known compound that binds a kinase protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to preferentially bind to or modulate the activity of a kinase target molecule.

In the above-mentioned assays, it may be desirable to immobilize either a kinase protein or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/kinase fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione-derivatized microtitre plates, which are then combined with the test compound or the test compound and either the nonadsorbed target protein or kinase protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components and complex formation is measured either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of kinase binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either kinase protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated kinase molecules or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96-well plates (Pierce Chemicals). Alternatively, antibodies reactive with a kinase protein or target molecules but which do not interfere with binding of the kinase protein to its target molecule can be derivatized to the wells of the plate, and unbound target or kinase protein

trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the kinase protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the kinase protein or target molecule.

In another embodiment, modulators of kinase expression are identified in a method in which a cell is contacted with a candidate compound and the expression of kinase mRNA or protein in the cell is determined relative to expression of kinase mRNA or protein in a cell in the absence of the candidate compound. When expression is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of kinase mRNA or protein expression. Alternatively, when expression is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of kinase mRNA or protein expression. The level of kinase mRNA or protein expression in the cells can be determined by methods described herein for detecting kinase mRNA or protein.

In yet another aspect of the invention, the kinase proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (*see*, e.g., U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Bio/Techniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and PCT Publication No. WO 94/10300), to identify other proteins, which bind to or interact with kinase protein ("kinase-binding proteins" or "kinase-bp") and modulate kinase activity. Such kinase-binding proteins are also likely to be involved in the propagation of signals by the kinase proteins as, for example, upstream or downstream elements of a signaling pathway.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as

polynucleotide reagents. For example, these sequences can be used to: (1) map their respective genes on a chromosome; (2) identify an individual from a minute biological sample (tissue typing); and (3) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

The isolated complete or partial kinase gene sequences of the invention can be used to map their respective kinase genes on a chromosome, thereby facilitating the location of gene regions associated with genetic disease. Computer analysis of kinase sequences can be used to rapidly select PCR primers (preferably 15-25 bp in length) that do not span more than one exon in the genomic DNA, thereby simplifying the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the kinase sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow (because they lack a particular enzyme), but in which human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes (D'Eustachio *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

Other mapping strategies that can similarly be used to map a kinase sequence to its chromosome include *in situ* hybridization (described in Fan *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries. Furthermore, fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase

chromosomal spread can be used to provide a precise chromosomal location in one step. For a review of this technique, *see* Verma *et al.* (1988) *Human Chromosomes: A Manual of* Basic *Techniques* (Pergamon Press, NY). The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results in a reasonable amount of time.

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, *Mendelian Inheritance in Man*, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, e.g., Egeland *et al.* (1987) *Nature* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the kinase gene can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

2. Tissue Typing

The kinase sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes and probed on a Southern blot to yield unique bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique for determining the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the kinase sequences of the invention can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The kinase sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. The noncoding sequence of SEQ ID NOS:1, 4, 7, 10, 13, or 16 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as in SEQ ID NOS:1, 3, 4, 6, 7, 9, 10, 12, 13, 15, 16 or 18 are used, a more appropriate number of primers for positive individual identification would be 500 to 2,000.

3. Use of Partial kinase Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. In this manner, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair, skin, or body fluids, e.g.,

blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" that is unique to a particular individual. As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to a non-coding region of SEQ ID NOS:1, 4, 7, 10, 13, or 16 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding region, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the kinase sequence or portions thereof, e.g., fragments derived from a noncoding region of SEQ ID NOS:1, 4, 7, 10, 13, or 16 having a length of at least 20 or 30 bases.

The kinase sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes that can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such kinase probes, can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, e.g., kinase primers or probes can be used to screen tissue culture for contamination (i.e., screen for the presence of a mixture of different types of cells in a culture).

C. Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trails are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. These applications are described in the subsections below.

1. Diagnostic Assays

One aspect of the present invention relates to diagnostic assays for detecting kinase protein and/or nucleic acid expression as well as kinase activity, in the context of a biological sample. An exemplary method for detecting the presence or absence of kinase proteins in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting kinase protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes kinase protein such that the presence of kinase protein is detected in the biological sample. Results obtained with a biological sample from the test subject may be compared to results obtained with a biological sample from a control subject.

"Misexpression or aberrant expression", as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes: expression at non-wild type levels, i.e., over or under expression; a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-transitional modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene, e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus.

A preferred agent for detecting kinase mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to kinase mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length or partial kinase nucleic acid, such as a nucleic acid of SEQ ID NOS: 1, 3, 4, 6, 7, 9, 10, 12, 13, 15, 16, 18, or a portion thereof, such as a nucleic acid molecule of at least 15, 30, 50, 100, 250, or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to kinase mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting kinase protein is an antibody capable of binding to kinase protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or $F(abN)_2$)can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

The term "biological sample" is intended to include tissues, cells, and biological fluids isolated from a subject, as well as tissues, cells, and fluids present within a subject. That is, the detection method of the invention can be used to detect kinase mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of kinase mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of kinase protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of kinase genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of kinase protein include introducing into a subject a labeled anti-kinase antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. Biological samples may be obtained from blood, serum, cells, or tissue of a subject.

The invention also encompasses kits for detecting the presence of kinase proteins in a biological sample (a test sample). Such kits can be used to determine if a subject is suffering from or is at increased risk of developing a disorder associated with aberrant expression of kinase protein. For example, the kit can comprise a labeled compound or agent capable of detecting kinase protein or mRNA in a biological sample and means for

determining the amount of a kinase protein in the sample (e.g., an anti-kinase antibody or an oligonucleotide probe that binds to DNA encoding a kinase protein, e.g., SEQ ID NOS: 1, 3, 4, 6, 7, 9, 10, 12, 13, 15, 16 or 18). Kits can also include instructions for observing that the tested subject is suffering from or is at risk of developing a disorder associated with aberrant expression of kinase sequences if the amount of kinase protein or mRNA is above or below a normal level.

For antibody-based kits, the kit can comprise, for example: (1) a first antibody (e.g., attached to a solid support) that binds to kinase protein; and, optionally, (2) a second, different antibody that binds to kinase protein or the first antibody and is conjugated to a detectable agent. For oligonucleotide-based kits, the kit can comprise, for example: (1) an oligonucleotide, e.g., a detectably labeled oligonucleotide, that hybridizes to a kinase nucleic acid sequence or (2) a pair of primers useful for amplifying a kinase nucleic acid molecule.

The kit can also comprise, e.g., a buffering agent, a preservative, or a protein stabilizing agent. The kit can also comprise components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples that can be assayed and compared to the test sample contained. Each component of the kit is usually enclosed within an individual container, and all of the various containers are within a single package along with instructions for observing whether the tested subject is suffering from or is at risk of developing a disorder associated with aberrant expression of kinase proteins.

2. <u>Prognostic Assays</u>

The methods described herein can furthermore be utilized as diagnostic or prognostic assays to identify subjects having or at risk of developing a disease or disorder associated with kinase protein, kinase nucleic acid expression, or kinase activity. Prognostic assays can be used for prognostic or predictive purposes to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with kinase protein, kinase nucleic acid expression, or kinase activity.

Thus, the present invention provides a method in which a test sample is obtained from a subject, and kinase protein or nucleic acid (e.g., mRNA, genomic DNA) is detected, wherein the presence of kinase protein or nucleic acid is diagnostic for a subject

having or at risk of developing a disease or disorder associated with aberrant kinase expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid cell sample, or tissue.

Furthermore, using the prognostic assays described herein, the present invention provides methods for determining whether a subject can be administered a specific agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) or class of agents (e.g., agents of a type that decrease kinase activity) to effectively treat a disease or disorder associated with aberrant kinase expression or activity. In this manner, a test sample is obtained and kinase protein or nucleic acid is detected. The presence of kinase protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant kinase expression or activity.

The methods of the invention can also be used to detect genetic lesions or mutations in a kinase gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion or mutation characterized by at least one of an alteration affecting the integrity of a gene encoding a kinase-protein, or the misexpression of the kinase gene. For example, such genetic lesions or mutations can be detected by ascertaining the existence of at least one of: (1) a deletion of one or more nucleotides from a kinase gene; (2) an addition of one or more nucleotides to a kinase gene; (3) a substitution of one or more nucleotides of a kinase gene; (4) a chromosomal rearrangement of a kinase gene; (5) an alteration in the level of a messenger RNA transcript of a kinase gene; (6) an aberrant modification of a kinase gene, such as of the methylation pattern of the genomic DNA; (7) the presence of a non-wild-type splicing pattern of a messenger RNA transcript of a kinase gene; (8) a non-wild-type level of a kinase-protein; (9) an allelic loss of a kinase gene; and (10) an inappropriate post-translational modification of a kinase-protein. As described herein, there are a large number of assay techniques known in the art that can be used for detecting lesions in a

kinase gene. Any cell type or tissue in which kinase proteins are expressed may be utilized in the prognostic assays described herein.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (*see*, e.g., U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (*see*, e.g., Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the kinase-gene (*see,* e.g., Abravaya *et al.* (1995) *Nucleic Acids Res.* 23:675-682). It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include self sustained sequence replication (Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.* (1988) *Bio/Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a kinase gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns of isolated test sample and control DNA digested with one or more restriction endonucleases. Moreover, the use of sequence specific ribozymes (*see*, e.g., U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in a kinase molecule can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin *et al.* (1996) *Human Mutation* 7:244-255; Kozal *et al.* (1996) *Nature Medicine* 2:753-759). In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the kinase gene and detect mutations by comparing the

sequence of the sample kinase gene with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Bio/Techniques* 19:448), including sequencing by mass spectrometry (*see*, e.g., PCT Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the kinase gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). *See*, also Cotton *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more "DNA mismatch repair" enzymes that recognize mismatched base pairs in double-stranded DNA in defined systems for detecting and mapping point mutations in kinase cDNAs obtained from samples of cells. *See*, e.g., Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662. According to an exemplary embodiment, a probe based on a kinase sequence, e.g., a wild-type kinase sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. *See*, e.g., U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in kinase genes. For example, single-strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild-type nucleic acids (Orita *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:2766; *see also* Cotton (1993) *Mutat. Res.* 285:125-144; Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis

to separate double-stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet.* 7:5).

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys. Chem.* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6230). Such allele-specific oligonucleotides are hybridized to PCR-amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele-specific amplification technology, which depends on selective PCR amplification, may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule so that amplification depends on differential hybridization (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3′ end of one primer where, under appropriate conditions, mismatch can prevent or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition, it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect

the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing prepackaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a kinase gene.

3. Pharmacogenomics

Agents or modulators that have a stimulatory or inhibitory effect on kinase activity (e.g., kinase gene expression) as identified by a screening assay described herein, can be administered to individuals to treat (prophylactically or therapeutically) disorders associated with aberrant kinase activity as well as to modulate the cellular growth, differentiation and/or metabolism. In conjunction with such treatment, the pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (e.g., drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of kinase protein, expression of kinase nucleic acid, or mutation content of kinase genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. *See*, e.g., Linder (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body are referred to as "altered drug action." Genetic conditions transmitted as single factors altering the way the body acts on drugs are referred to as "altered drug metabolism". These pharmacogenetic conditions

can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (antimalarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, a PM will show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of kinase protein, expression of kinase nucleic acid, or mutation content of kinase genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a kinase modulator, such as a modulator identified by one of the exemplary screening assays described herein.

4. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of kinase genes (e.g., the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening but also in clinical trials. For example, the effectiveness of an agent, as determined by a screening assay as described herein, to increase or decrease kinase gene expression, protein levels, or protein activity, can be monitored in clinical trials of subjects exhibiting decreased or increased kinase gene expression, protein levels, or protein activity. In such clinical trials, kinase expression or activity and preferably that of other genes that have been implicated in for example, a cellular proliferation disorder, can be used as a marker of cellular growth and differentiation.

For example, and not by way of limitation, genes that are modulated in cells by treatment with an agent (e.g., compound, drug, or small molecule) that modulates kinase activity (e.g., as identified in a screening assay described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of kinase genes and other genes implicated in the disorder. The levels of gene expression (i.e., a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of kinase genes or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (1) obtaining a preadministration sample from a subject prior to administration of the agent; (2) detecting the level of expression of a kinase protein, mRNA, or genomic DNA in the preadministration sample; (3) obtaining one or more postadministration samples from the

subject; (4) detecting the level of expression or activity of the kinase protein, mRNA, or genomic DNA in the postadministration samples; (5) comparing the level of expression or activity of the kinase protein, mRNA, or genomic DNA in the preadministration sample with the kinase protein, mRNA, or genomic DNA in the postadministration sample or samples; and (vi) altering the administration of the agent to the subject accordingly to bring about the desired effect, i.e., for example, an increase or a decrease in the expression or activity of a kinase protein.

C. Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant kinase expression or activity. "Subject", as used herein, can refer to a mammal, e.g., a human, or to an experimental or animal or disease model. The subject can also be a non-human animal, e.g., a horse, cow, goat, or other domestic animal. Additionally, the compositions of the invention find use in the treatment of disorders described herein.

"Treatment" is herein defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease. A "therapeutic agent" includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject a disease or condition associated with an aberrant kinase expression or activity by administering to the subject an agent that modulates kinase expression or at least one kinase gene activity. Subjects at risk for a disease that is caused, or contributed to, by aberrant kinase expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the

kinase aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of kinase aberrancy, for example, a kinase agonist or kinase antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating kinase expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of kinase protein activity associated with the cell. An agent that modulates kinase protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of a kinase protein, a peptide, a kinase peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more of the biological activities of kinase protein. Examples of such stimulatory agents include active kinase protein and a nucleic acid molecule encoding a kinase protein that has been introduced into the cell. In another embodiment, the agent inhibits one or more of the biological activities of kinase protein. Examples of such inhibitory agents include antisense kinase nucleic acid molecules and anti-kinase antibodies.

These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a kinase protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or a combination of agents, that modulates (e.g., upregulates or downregulates) kinase expression or activity. In another embodiment, the method involves administering a kinase protein or nucleic acid molecule as therapy to compensate for reduced or aberrant kinase expression or activity.

Stimulation of kinase activity is desirable in situations in which a kinase protein is abnormally downregulated and/or in which increased kinase activity is likely to have a beneficial effect. Conversely, inhibition of kinase activity is desirable in situations in

which kinase activity is abnormally upregulated and/or in which decreased kinase activity is likely to have a beneficial effect.

EXPERIMENTAL

Example 1: Recombinant Expression of kinase sequences in Bacterial Cells

In this example, the kinase sequence is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, the kinase sequence is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-kinase fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

Example 2: Expression of Recombinant Kinase Protein in COS Cells

To express the kinase gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire kinase protein and an HA tag (Wilson *et al.* (1984) *Cell* 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the kinase DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the kinase coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the kinase coding sequence. The PCR amplified fragment and the

pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen are different so that the kinase gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5α, SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the kinase-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the kinase polypeptide is detected by radiolabelling (^{35}S-methionine or ^{35}S-cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labeled for 8 hours with ^{35}S-methionine (or ^{35}S-cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the kinase coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the kinase polypeptide is detected by radiolabelling and immunoprecipitation using a kinase specific monoclonal antibody.

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.